Communal Nursing Decisions and Social Preferences in Wild House Mice (*Mus musculus domesticus*)

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SUMMARY

Sociality between same-sex conspecifics can lead to a variety of benefits including increased offspring survival, increased group cohesion, decreased stress and increased life span. Same-sex individuals will often form social bonds or preferences within groups in order to profit from these fitness advantages. Females commonly form social preferences during the collective care of offspring that results in two or more females equally contributing towards raising the combined offspring, an activity that is considered costly and open to cheating. Therefore, choosing a suitable partner to avoid cheating and gain mutual benefits should be important. In this study, I analysed social partner choice in female house mice (*Mus musculus domesticus*). Female house mice regularly communally nurse whereby two or more females pool their litters in the same nest and nurse all offspring present. When communal nursing occurs with a specific partner it can result in increased lifetime reproductive success. Therefore, I aimed to determine what factors influence the choice of a communal nursing partner, and whether female house mice form social preferences.

First, I investigated communal nursing decisions in free-living house mice to determine if females choose to communally nurse or whether it was a by-product of sharing the same nesting sites. Number of available partners varied (another female with pups sharing the same group and home area), however, female's chose to nurse communally when there were more potential partners available, suggesting there was a higher chance of a preferred partner being available. Females also preferred to communally nurse with another female when her partners pups were young (there was a small age difference between the two litters). In addition, I found that a female shared her home area with other females that were genetically similar to them, suggesting they shared nesting sites with related females. This further suggests that communal nursing occurs among groups of related females, which would lead to increased indirect fitness benefits. In a second step, I aimed to verify the existence of social preferences among free-living female house mice, and found that females socialised more often with females that were more related to them. Within these associations females had four female partners they preferred (spent the most time with), and these partners were relatively consistent from one month to the next. These results demonstrate that female house mice do form social preferences for specific female partners, and have relatively stable female groups.

In the third study, my aim was to understand the proximate mechanisms that may underlie same-sex social preference. A key candidate was the endocrinological system, more specifically the peptide hormone oxytocin, as it has been linked to a range of social and cooperative behaviours. Therefore, I was interested to understand whether oxytocin influenced the initial behaviours a female exhibited towards an unfamiliar female, and determine if oxytocin could lead to a preference being established. I found oxytocin-treated females did not differ from saline-treated females in their social behaviours, but when the oxytocin females were given the choice between their partner and a new stranger they demonstrated no preference for their partner. This suggested that oxytocin acted to hinder the formation of a preference between these females. In a final step, I aimed to determine whether oxytocin would influence a pair's ability to cooperate by communal nursing. I found that oxytocin-treated females took longer to successfully raise a communal litter, suggesting oxytocin had a negative effect on the females early social relationship that led to a reduced tendency for reproductive cooperation. The findings suggest that oxytocin does not appear to facilitate initial behaviours and familiarity between unfamiliar females. Therefore, previous familiarity between females could be of importance when investigating oxytocin's effect on preference and cooperation in female house mice.

Overall this thesis highlights that female house mice use a range of processes when deciding whether to communally nurse. The findings suggest that familiarity and relatedness are important for communal nursing to occur, and that previously well documented factors, such as oxytocin appear not to facilitate cooperation or preference formation among unrelated, unfamiliar female pairs. Research such as this highlights the importance of multiple testing, and the need to verify findings using both empirical and free-living studies. Importantly, I provide a foundation for future studies to expand on our knowledge of communal nursing and its implications.

ZUSAMMENFASSUNG

Sozialverhalten unter gleichgeschlechtlichen Artgenossen kann viele Vorteile haben, wie zum Beispiel verbesserte Überlebenswahrscheinlichkeit der Nachkommen, verbesserter Zusammenhalt in der Gruppe, verringerter Stress oder eine längere Lebensdauer. Innerhalb von Gruppen werden als Voraussetzung für solche Fitness-Vorteile häufig soziale Bindungen oder Präferenzen unter den Gruppenmitgliedern beobachtet. Weibchen bilden soziale Präferenzen oft zur gemeinschaftlichen Jungenaufzucht, bei der zwei oder mehr Mütter gleichermassen zur Betreuung aller Nachkommen beitragen. Diese Form der Jungenaufzucht gilt als kostspielig und Betrug anfällig. Daher sollte die Wahl einer geeigneten Partnerin wichtig sein, um ein Ausgenutztwerden zu vermeiden und um von mutualistischen Vorteilen zu profitieren. In meiner Dissertation habe ich mich auf die Wahl von Sozialpartnerinnen bei weiblichen Hausmäusen (*Mus musculus domesticus*) konzentriert. Hausmausweibchen ziehen ihre Würfe regelmässig gemeinschaftlich auf, wobei zwei oder mehr Weibchen alle Jungen im selben Nest säugen. Gemeinschaftliche Jungenaufzucht kann den Lebensfortpflanzungserfolg eines Weibchens erhöhen, wenn es mit einem bestimmten, richtigen Partner kooperiert. Das Ziel meiner Studien war, die Faktoren zu analysieren, welche die Wahl einer Partnerin zur gemeinschaftlichen Jungenaufzucht beeinflussen und zu klären, ob Hausmausweibchen soziale Präferenzen für Partnerinnen entwickeln.

Als erstes habe ich das Verhalten von Weibchen in einer frei lebenden Population untersucht und überprüft, ob es Hinweise auf Sozialpartnerwahl bei der gemeinschaftlichen Jungenaufzucht gibt, oder ob diese ein Nebenprodukt des Gruppenlebens (gemeinsame Nestnutzung) ist. Die Anzahl möglicher Partner (andere Weibchen mit Nestjungen im selben Aufenthaltsbereich) variierte. Dennoch zeigten Weibchen umso häufiger gemeinsame Jungenaufzucht, je mehr Optionen vorhanden waren, was auf zunehmende Chance für das Vorhandensein einer geeigneten Partnerin hinweist. Weibchen bevorzugten zur gemeinschaftlichen Jungenaufzucht eine Partnerin mit einem jungen Wurf, so dass der Altersunterschied zwischen den Würfen gering war. Interessanterweise lebten die Weibchen in der untersuchten Population in einer genetisch strukturierten Umgebung, in räumlicher Nähe mit genetisch ähnlichen Weibchen. Dies bedeutet, dass Hausmausweibchen ihre Nester häufig mit verwandten Weibchen teilen, was die Evolution von Kooperation durch indirekte Fitnessgewinne erleichtern sollte. Je höher die mittlere Verwandtschaft innerhalb einer Gruppe war, desto häufiger kooperierten auch die Weibchen. In einem nächsten Schritt habe ich das Vorhandensein von sozialen Präferenzen unter den frei lebenden Mäusen überprüft und konnte zeigen, dass Weibchen mit näher verwandten Partnerinnen mehr Zeit im selben Nest verbrachten, was für eine stärkere soziale Bindung spricht. Innerhalb einer sozialen Gruppe hatten Weibchen typischerweise vier bevorzugte Sozialpartnerinnen (mit denen sie die meiste Zeit verbrachten), und diese Partnerschaften blieben über einige Monate bestehen. Hausmausweibchen leben folglich in relativ stabilen Gruppen und etablieren soziale Präferenzen für bestimmte, meist verwandte Partnerinnen.

Im dritten Projekt untersuchte ich experimentell proximate Mechanismen, welche die Grundlage für die Präferenz einer Sozialpartnerin sein können. Ein Hauptanwärter ist das Hormonsystem, genauer, das Peptidhormon Oxytocin, da es mit einer Reihe sozialer und kooperativer Verhalten in Zusammenhang gebracht wird. Ich wollte folglich testen, ob Oxytocin das Verhalten zwischen unbekannten Weibchen, die sich erstmals treffen, beeinflusst und zur Ausbildung einer Präferenz zur Partnerin (einer Paarbindung) beiträgt. Mit Oxytocin behandelte Weibchen unterschieden sich in ihren sozialen Interaktionen nicht von Weibchen der Kontrollgruppe, allerdings zeigten sie im Unterschied zur Kontrollgruppe keine Präferenz für die Partnerin, wenn sie die Wahl zwischen dieser und einem unbekannten Weibchen hatten. Oxytocin verhinderte demnach die Ausbildung einer Bindung oder Präferenz zwischen den Weibchen. In einem weiteren Schritt habe ich folglich wiederum in einem Verhaltensversuch getestet, ob Oxytocin die Kooperationsbereitschaft bei der gemeinschaftlichen Jungenaufzucht beeinträchtigt. Mit Oxytocin behandelte Weibchen benötigten tatsächlich länger, bis sie erfolgreich einen gemeinschaftlichen Wurf aufzogen. Als Grund kann mangelnde soziale Bindung während der Anfangsphase der Begegnung unter den Weibchen angenommen werden,

was die Kooperation erschwert. Diese Versuche zeigen, dass Oxytocin nicht das Bekanntwerden und die Vertrautheit unter zwei zuvor unbekannten Weibchen fördert. Die vorhergehende Vertrautheit zwischen Weibchen könnte allerdings entscheidend dafür sein, dass Oxytocin die Sozialpartnerpräferenz und das Kooperationsverhalten von weiblichen Hausmäusen beeinflusst.

Insgesamt belegen die im Rahmen meiner Dissertation gesammelten Daten, dass Hausmausweibchen für die Entscheidung, ob sie einen Wurf alleine oder gemeinsam mit einer Partnerin aufziehen, eine Reihe unterschiedlicher Prozesse nutzten Bekanntschaft (Vertrautheit) und Verwandtschaft begünstigten die gemeinschaftliche Jungenaufzucht. Ein zuvor gut dokumentierter Einflussfaktor auf das Sozialverhalten, Oxytocin, förderte dagegen nicht die Präferenzbildung und Kooperation unter zuvor unbekannten, genetisch nicht-verwandten Weibchen. Diese Studien belegen den Wert der Überprüfung von wissenschaftlichen Ergebnissen durch unabhängige Untersuchungen und die Notwendigkeit, Resultate sowohl experimentell als auch anhand des Studiums von Tieren in ihrer natürlichen Umgebung zu verifizieren. Meine Studien beantworten wichtige Fragen und liefern weiterhin die Grundlage für zukünftige Forschung, um unser Wissen über gemeinschaftliche Jungenaufzucht und dessen Folgen zu vertiefen.

General Introduction

GENERAL INTRODUCTION

Sociality is thought to evolve when the benefits gained from forming close associations with conspecifics are greater than the costs an individual incurs (Krause and Ruxton 2002; Silk 2007a). Living in a social group can have many costs such as increased disease transfer, competition over mating or social partners, risk of losing offspring to infanticidal conspecifics, or increased predation risk (reviewed in: Silk 2007a). On the other hand, such costs can be outweighed by the benefits gained from sociality, including decreased predation risk, assistance in finding or defending of mates and territories, and cooperation when rearing offspring or when foraging (Clutton-Brock 2006; Silk 2007a; Shultz and Dunbar 2010; Seyfarth and Cheney 2012). Sociality has independently arisen in many vertebrate and some invertebrate groups (Choe and Crespi 1997; Cockburn 1998; Silk 2007a; Shultz and Dunbar 2010), and such social groups can be composed of small pair-bonded units or larger aggregations of individuals (Silk 2007a). Within group-living species interactions between conspecifics occur on a frequent basis presenting opportunities for individuals to help each other (Silk 2014), and for females living in social groups cooperation can result in improved reproduction (Mumme et al. 1988; Clutton-Brock 2002; König 2006; Silk 2014). Such cooperation has commonly been studied in cooperative breeders, where helpers assist in the care of the dominant pairs offspring, or in communal breeders, where all individuals care for their collective offspring (Packer et al. 1992; König 1997; Clutton-Brock 2006). Evidence from primate studies have revealed that long-term social partnerships with same-sex group members can result in fitness benefits (Silk 2007a; 2007b; 2014). For example, Silk et al. (2003) observed that among female savannah baboons, *Papio cynocephalus*, social integration was positively correlated with infant survival, and in a separate study, having strong and consistent partnerships led to increased longevity (Silk. et al 2010a). These studies therefore emphasise the importance of individualised interactions between females in social groups, and that choosing the correct social partner should be important. Such a scenario involving choice coincides with the idea of social selection raised by West-Eberhard (1979; 1983), whereby choice of social partner is expected to result in the evolution of specific traits that allow an individual to gain fitness benefits through successful social interactions (West-Eberhard 1979; 1983; Wolf et al. 1999). Thus, to understand female-female social interactions it is important to study the existence of individual preferences or social bonds, and the factors used by females to choose among different same-sex partners.

Formation of Social Preferences

Same-sex individuals will often form social bonds that are behaviourally expressed as individual preferences. A preference can be a short association, based on a specific or preferred trait such as during social or sexual selection, or a long-term affiliative relationship (Silk et al. 2003; 2009; Shultz and Dunbar 2010; Roughgarden 2012; Seyfarth and Cheney 2012). Nevertheless, a preference based on a specific trait may also be the foundation for a long-term affiliative relationship. Kinship is often considered the driving force behind social preferences, and in order to benefit from fitness advantages an individual should direct its social and cooperative interactions towards kin (Hamilton 1964a; 1964b). Interactions between close relatives have been observed in a range of species, including male chimpanzees, *Pan troglodytes*, that formed equitable and long lasting bonds with maternal kin (Mitani 2009), female spotted hyena, *Crocuta crocuta*, associated more frequently with adult female kin than non-kin (Holekamp et al. 2012), and adult female baboons were shown to bias their behaviour towards maternal and paternal half sisters (Smith et al. 2003). Nevertheless, studies have shown

that individuals will form preferences for unrelated partners that can also be adaptive (Weidt et al. 2008; Cameron et al. 2009; Frére et al. 2010; Schülke et al. 2010), suggesting that the mechanisms behind choosing a social partner and establishing social preferences are not entirely dictated by kinship. Therefore, the formation of social preferences or bonds may rely on a number of different processes or specific attributes of a partner.

Social relationships may be beneficial in different ways, however, the factors that motivate individuals to form these preferences should also be considered (Massen et al. 2010). Social bonds and social interactions can be characterised by repeated physical contact and positive social stimuli between individuals (Uvnas-Moberg 1998), which may result in the facilitation of sociality and the motivation of individuals to affiliate (Campbell 2008). The facilitation or formation of social preferences have been linked to endocrinological mechanisms, in particular that of the oxytocinergic system (Massen et al. 2010; Anacker and Beery 2013; Wittig et al. 2014). Oxytocin, a peptide hormone, has been connected to a range of social and cooperative behaviours in both humans and animals, these include the facilitation of bonds between a mother and her offspring, and between mating partners (McCarthy 1990; Witt et al. 1990; Carter et al. 1992; Popik et al. 1992; Smith et al. 2010; Anacker and Beery 2013; Crockford et al. 2013; 2014; Mooney et al. 2014; Wittig et al. 2014; Carter and Wilkinson 2015). In particular, oxytocin has been extensively studied for its effects on pair bonding in the monogamous prairie vole, *Microtus ochrogaster*, where it was demonstrated to play a role in facilitating pre-copulatory bonding between males and females (Williams et al. 1994; Insel and Hulihan 1995; Cho et al. 1999; Ross & Young 2009). Oxytocin was further found to be elevated after positive social interactions with conspecifics, such as grooming with a preferred social partner (Crockford et al. 2013), and food sharing among conspecifics (Wittig et al. 2014). Although studies have predominately focused on the positive influences of oxytocin, there are also indications that oxytocin can enhance negative social perceptions (Declerck et al. 2010; Mikolajczak et al. 2010; De Dreu 2012; Crockford et al. 2014; Beery 2015). The research surrounding oxytocin is fairly conflicting and results can often be dependent on the circumstances, the presence of specific social partners, and the species being studied (Bartz et al. 2010; Crockford et al. 2013; 2014; Wittig et al. 2014; Beery 2015). Consequently, this suggests that there are many aspects that need to be considered when investigating the mechanisms behind choice of social partner.

Consequences of Social Partner Preferences

While social preferences between same-sex partners has been observed in a range of taxa (Möller et al. 2006; Silk et al. 2006; Langergraber et al. 2009; Lehmann and Boesch 2009; Mitani 2009; Cheney et al. 2012; Carter et al. 2012; Best et al. 2013), research on the form and function of such preferences is fairly limited (Silk 2014). Studies assessing the outcome of close social bonds on health, life span and reproductive success of individuals within social groups have predominately focused on primates. Silk (2014) highlighted that this taxonomic bias in research on social bonds demonstrates that primatologists have had a long-term interest in the complex dynamics of social relationships. However, conspecifics are considered preferred partners in a range of other species, and in the presence of both kin and non-kin individuals can benefit from a variety of fitness advantages (Packer et al. 1991; König 1994a; Feh 1999; Silk 2007a; Weidt et al. 2008; Cameron et al. 2009; Schülke et al. 2010).

Same-sex social preferences occur between both male and female dyads, and are formed in the context of offspring care, or during coalitions and alliances (Packer et al. 1991; Connor et al. 1992; Silk et al. 2003; 2006; 2010b; Langergraber et al. 2007; Weidt et al. 2008;

Cameron et al. 2009; Langergraber et al. 2009; Mitani 2009; Schülke et al. 2010). However, female philopatry can increase the possibility that cooperative relationships arise, and may therefore facilitate the formation of social preferences (see also Silk 2007a; Seyfarth and Cheney 2012). Hence, studies have investigated the advantages of social bonds or preferences among females, these include increased offspring survival, longevity, decreased stress and increased group cohesion (König 1994a; Silk 2007b; Yee et al. 2008; Shultz and Dunbar 2010; Silk et al. 2010a; Silk 2011; Seyfarth and Cheney 2012). Positive correlates between fitness benefits and female preferences have been documented in a variety of species, such as female house mice, *Mus musculus domesticus,* which had greater reproductive success when nursing with a preferred female partner (Weidt et al. 2008). In primates, Silk et al. (2003) observed that between female savannah baboons social integration was positively correlated with infant survival, female horses, *Equus equus,* that were more socially integrated had higher foal birth and survival rates (Cameron et al. 2009), and calving success in female dolphins, *Tursiops sp.*, was dependent on the strength of their social relationships with other females (Frére et al. 2010). Taken together, these studies demonstrate the benefits of sociality between same-sex conspecifics. They further highlight the importance of social partnerships in achieving the observed fitness advantages, and that the structure and quality of social relationships may play a role in an individual's capacity to enhance benefits and reduce the costs associated with social living (Silk 2014).

Choice of a social partner is believed to be especially important during the collective care of offspring, which often results in joint nesting and an equal contribution towards raising the combined offspring (Packer et al. 1992; König 1997; 2006; Hayes 2000). Collective care of offspring can even encompass the non-selective nursing (via lactation) of young produced by another female within a social group (Packer et al. 1992; König 1997; 2006). Nursing of non-offspring is considered a costly activity and can be open to exploitation (Palanza et al. 2005; Ferrari et al. 2015; 2016; Schmidt et al. 2015), therefore choosing a suitable partner to avoid exploitation and gain mutual benefits should be important (Weidt et al. 2008; König and Lindholm 2012). Rodents, in particular female house mice, use a specific form of collective care known as communal nursing (Packer et al. 1992; König 1997; Hayes 2000), where females pool their litters in the same nest and indiscriminately nurse all offspring present (König 2006). Cooperation through communal nursing not only leads to increased lifetime reproductive success (König 1994a; 1994b), but recent evidence suggests that communal nursing may not be a by-product of sharing the same nesting sites as it was previously believed (Weidt et al. 2014). Therefore, house mice offer an ideal study species to investigate the choices behind communal nursing decisions, the aspects influencing preference formation, and whether females form social preferences.

AIMS OF THE STUDY

Communal nursing of house mice is a key example of cooperation where females pool litters in the same nest and indiscriminately nurse own and non-offspring (König 2006). The direct fitness benefits associated with communal nursing shown in laboratory studies suggest it to be a selected component of female house mice reproductive behaviour. However, such cooperation can be open to cheating (Ferrari et al. 2015; 2016), and previous studies have illustrated the importance of social partner choice or preference in stabilising cooperation (König 1994b; 2006; Weidt et al. 2008). However, there is surprisingly little known about communal nursing in wild mice and the aspects that influence communal nursing decisions. I therefore, aimed to analyse partner choice and potential partner preferences among females by

combining results from behavioural and genetic analysis of a long-term study on free-living house mice, with experiments under standardized laboratory conditions on mice derived from the same population.

STUDY SPECIES

House mice are plural breeders that live in social groups comprised of a dominant male, some subordinate males, and a number of breeding and non-breeding females (Anderson and Hill 1965; Lidicker 1976; MacKintosh 1981; König and Lindholm 2012). Mice are a small territorial rodent, whereby males compete over territories for access to breeding opportunities (Defries and McClearn 1970; vom Saal and Howard 1982), and females compete over access to nesting sites (vom Saal et al. 1995; König and Lindholm 2012; Stockley et al. 2013). Females give birth to altricial pups that require maternal care until weaning (König and Markl 1987), and it is common that two or more females will cooperate by pooling their litters in one nest, and nursing all the pups in a communal litter non-selectively (König 2006). When pups are pooled in a communal litter females are unable to distinguish own pups from those of another female that has pups in the same nest (König 1989a; 1989b; Hager and Johnstone 2004), or may have only very limited abilities to do so (Hager and Johnstone 2005). Therefore, females with pups in a communal litter invest in the care of all pups in the nest, through thermoregulation, nursing of the young and nest defence (König 1997; Auclair et al. 2014; Ferrari et al. 2015; 2016). Female house mice may also use solitary nursing in which a female will nurse her pups alone. In the laboratory, females that nursed communally were observed to have greater reproductive success than those that nursed solitarily (König 1994b; Manning et al. 1995), and reproductive success was found to be highest when females were paired with a related, familiar or preferred female partner (König 1994a; 1994b; Weidt et al. 2008). Therefore, communal nursing is believed to be a key form of cooperation and a selective componant of female house mice reproductive behaviour.

Study System

The data presented for the first two chapters of my thesis were collected from a free-living population of house mice situated in an old barn close to Zurich, Switzerland. This population has been studied since 2002. I used life history and demographic data, as well as social interaction data collected by an antenna system (first installed in 2007), to investigate communal nursing decisions and social preferences in female mice. My study focused on the data collected from the barn during the entirety of 2008 and 2009. For detailed descriptions of the barn set up, data collection and antenna system please see: methods in chapter 1 and 2, as well as König and Lindholm (2012); Auclair et al. (2014); and König et al. (2015). In my two remaining chapters, I carried out controlled laboratory studies using mice derived from the barn population (F1 to F3 generation of mice bred under otherwise controlled laboratory breeding conditions).

THESIS OUTLINE

The main objective of my thesis was to examine communal nursing decisions and social preferences in female house mice. A combination of methods were undertaken to assess these objectives, using both data from a free-living population of house mice and controlled laboratory experiments.

In **chapter 1,** I investigated whether communal nursing by female house mice is a selected trait, which would suggest a preference or a decision, and is not a consequence of sharing the same nesting sites. I used a long-term data set collected on a free-living population of house mice. Population density of the study was in the range of previous studies, and can be considered as neither high nor low, with maximally 2.6 adult mice per square metre. I analysed a two-year period when data were collected by an automatic antenna recording system that allowed analysis of behavioural data using individual nest box use and meetings between individuals in these nest boxes. I focused on situations in which females had at least one option (another female with pups) to nurse communally in her home area, and could decide whether to nurse communally or solitarily. Such situations should reveal whether females were discriminatory in their choice of communal nursing partner. In addition, I considered different characteristics of a focal female's potential partners and their litters (for example; pairwise relatedness, age difference, association time, age of the pups and litter size) to gain information on the possible factors involved in choosing a communal nursing partner.

Given that communal nursing appears to reflect social partner choice in female house mice, and that association time among females during pregnancy or even earlier is important, I focused in **chapter 2** on whether females establish individualised affiliative associations within social groups, and if these associations were stable. Again, I used the long-term data and focused on the frequency and duration of meetings between female dyads within the same home area. I used a sociality index established for primates, which created a measure to assess the strength of a dyad's affiliative relationship. With this sociality index I could rank each dyad from highest to lowest, and determine whether female's associated more often with certain partners, and if these partners were stable over time. Given the importance of relatedness in the evolution of social behaviour and in stabilising the risk of exploitation, I also included pairwise relatedness among female partners.

In the remaining two chapters of my thesis I was interested to understand the proximate mechanisms that may underlie intra-sexual preference formation. Given that association time is believed to be important, a key candidate was the endocrinological system, more specifically the peptide hormone oxytocin, as it has been linked to a range of social and cooperative behaviours including the facilitation of bonds between a mother and her offspring and between mating partners (reviewed in: Anacker & Beery, 2013). In **chapter 3** I tested, under laboratory conditions, whether administration of exogenous oxytocin influenced the initial behaviours a female exhibited towards an unfamiliar female, and determined if oxytocin could facilitate the establishment of a preference. Finally, **chapter 4** examined whether treatment with oxytocin at a time when females potentially establish a social relationship/preference would influence their later cooperative propensity to communally nurse offspring. Oxytocin was experimentally increased in pairs of females during a 3-day cohabitation period, after which a male was introduced. Latency until weaning of a successful communal litter (where both females gave birth, pooled their litters and at least one pup was weaned from each litter) was assessed and compared against saline treated control females, to determine how oxytocin influenced the females tendency to cooperate.

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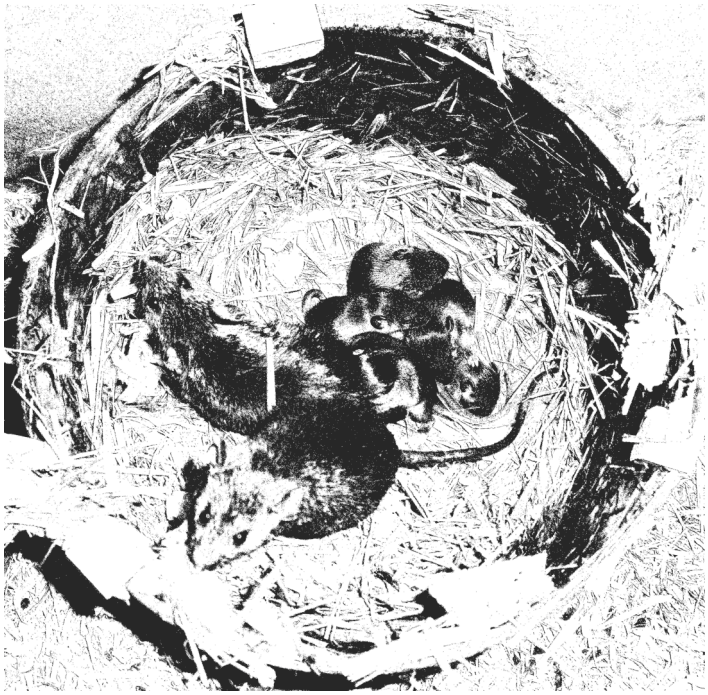
CHAPTER 1

Females Choose Familiar Communal Nursing Partners with Young Pups from a Group of Genetically Similar Females

To be submitted



Females Choose Familiar Communal Nursing Partners with Young Pups from a Group of Genetically Similar Females

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ABSTRACT

Communal nursing in house mice is an example of cooperation where females pool litters in the same nest and indiscriminately nurse own and non-offspring. The direct fitness benefits associated with communal nursing shown in laboratory studies suggest it to be a selected component of female house mice reproductive behaviour. However, past studies on communal nursing in free-living populations have often debated whether it is a consequence of sharing the same nest or an active choice. Here using data from a long-term study of free-living, wild house mice we investigated individual nursing decisions and determined what factors influenced a female's decision to nurse communally. We found that females chose to nurse solitarily more often than expected by chance, but the likelihood of nursing solitarily decreased when females had more partners available. While finding no influence of pairwise relatedness on partner choice, we found females shared their social environment with genetically similar individuals, suggesting a female's home area consisted of related females. Within such a home area females preferred to nest communally when the general relatedness of her available options was higher, and form a communal litter with a female that was familiar and had young pups. Our findings suggest that communal nursing was not a by-product of sharing the same nesting sites, but females choose communal nursing partners from a group of genetically similar females, and ultimately the decision may then depend on the pool of options available.

INTRODUCTION

The collective care of offspring is a key form of cooperation where individuals care for the offspring of others, and in doing so gain direct and/or indirect fitness benefits (Jennions and Macdonald 1994; König 1997; Clutton-Brock 2002; Silk 2007). Within an evolutionary framework such investment into caring for the offspring of conspecifics has been studied in a diverse range of taxa, including social insects (Choe and Crespi 1997), birds (Cockburn 1998) and mammals (Clutton-Brock 2006). A specific type of collective care is communal nursing where two or more reproducing females pool their litters in the same nest or burrow and indiscriminately nurse own and non-offspring (Packer et al. 1992; Manning et al. 1995; Hayes 2000; König 2006). Recent evidence has demonstrated the potential for cheating during communal nursing (Ferrari et al. 2016), which suggests that choice of communal nursing partner is important. However, the mechanisms behind choice of partner within the context of cooperative care are poorly understood.

One species where communal nursing has been studied extensively in the laboratory, and less so in the wild, are house mice (*Mus musculus domesticus*). Laboratory studies have demonstrated that two females regularly establish egalitarian relationships in which they nurse each other's pups non-selectively (König and Markl 1987; König 1994a; Hayes 2000; Ferrari et al. 2015). Females pool their litters in a single nest, and for an extended period of time (up to 3 weeks) invest in all pups present. Additionally, when pups are pooled in the same nest females with pups already present are unable to distinguish own from alien offspring before the onset of weaning (Chantrey and Jenkins 1982; König 1989; Manning et al. 1995; Ferrari et al. 2016). In consequence, communal nursing has been argued to be a side effect of sharing the same social environment or nesting sites (Manning et al. 1995; Hayes 2000). In a similar vein, communal nursing has been associated with high population densities and a lack of dispersal opportunities (Wolff 1994).

Conversely, Weidt et al. (2014) found in a free-living population that females nest solitarily despite having up to five potential communal nursing partners (another female with pups in their social environment), suggesting there is an element of choice in communal nursing decisions. In laboratory experiments, communal nursing has been shown to provide fitness advantages for females, such as increased lifetime reproductive success in comparison to solitary nursing. Females achieved higher success when they nursed with a related, familiar or preferred female partner (König 1994b; 1994a; Weidt et al. 2008). Individual lifetime reproductive success, however, decreased below that of a solitary nursing female when groups of three or more females pooled their litters (König 2006), suggesting the optimal communal nursing group size to be two females. Additionally, pups from communal litters had higher survival probabilities (Manning et al. 1995; Auclair, König, and Lindholm 2014), but only when offspring in the communal litter had different fathers, suggesting that the pups were better protected against male infanticide (Auclair, König, and Lindholm 2014). Females can also benefit from increased foraging time or time away from the nest, without affecting the amount of maternal care received by the offspring (Auclair, König, Ferrari, et al. 2014). Furthermore, communal nursing may have thermoregulatory benefits, allowing pups to allocate more resources to growth, and therefore is expected to occur more frequently at higher altitudes and lower temperatures (reviewed in: Hayes 2000).

Communal nursing can therefore be considered adaptive, and if this is the case it raises the question of whether females use specific factors of a partner and/or their litters when deciding to communally nurse. The importance of choosing a communal nursing partner in house mice is supported by recent empirical evidence suggesting a potential for exploitation

during communal nursing (Ferrari et al. 2015; 2016). Since females indiscriminately nurse litters and produce milk according to the total number of pups in the communal litter and not just own litter size, a female with a smaller litter in the communal nest would overinvest in relation to her own litter size, which would benefit the partner (Ferrari et al. 2015). Furthermore, when the difference in birth litter size was experimentally increased, females were less inclined to nurse communally (Ferrari et al. 2016). According to theoretical models and empirical evidence, exploitation costs are more often tolerated among relatives due to the indirect benefits gained (West et al. 2002; Mathot and Giraldeau 2010). Such findings suggest that relatedness should be important in communal nursing decisions, and evidence in support of this has indicated that females typically nest with kin (Wilkinson and Baker 1988), and prefer partners that share allelic forms of the major histocompatibility complex (MHC) gene (Manning et al. 1992). Green et al. (2015) further revealed that female house mice choose nesting partners who are closely related, and those who share own major urinary protein (MUP) genotype. On the other hand, females can establish successful cooperative relationships with previously unfamiliar, unrelated partners (König 1994b; Weidt et al. 2008), suggesting that other factors can allow for effective communal nursing (König 1994b; Dobson et al. 2000; Weidt et al. 2008). Taken together these findings imply that females may use specific cues to assess factors of potential communal nursing partners and/or their litters. Weidt et al. (2008) further demonstrated that female house mice have increased lifetime reproductive success when nursing with a preferred female partner, suggesting that not any partner is suitable. Therefore, selection on choice of social partner is expected to result in the evolution of specific traits that allow its bearer to gain fitness benefits through successful social interactions (West-Eberhard 1979; 1983; Wolf et al. 1999). However, in female house mice, to understand the role of partner choice, we first need to analyse the factors involved in the decision to nurse communally.

In the present study, we investigated in detail communal nursing decisions in the natural, complex social environment of free-living house mice. Analysis of communal nursing decisions was carried out post-hoc and did not involve manipulation of the study population. We specifically focused on situations in which a female had at least one potential nursing partner. Over a period of two years, we collected information on a female's potential nursing partners and their litters to investigate whether communal nursing was a by-product of sharing the same nesting sites or an active decision. We looked at whether females always nursed communally when they had the opportunity to do so, and tested our data against a null model to determine whether or not female decisions were independent of nest occupancy. We further tested if choice is linked to seasonal effects on reproduction, population size, or number of nest sites used by the focal female. As previous studies have found that solitary nursing occurred in about 67 % of litters (Weidt et al. 2014), we expected females to choose options that would avoid exploitation, allowing for mutual benefits (König 1994a; König and Lindholm 2012). We predicted that a female should preferentially join another female when: a) the other litter was solitary; b) the other female did not have a larger number of pups to herself minimising the risk of exploitation (however our measure of litter size in this study may not be fully representative of birth litter size); and c) the other female was kin. Female house mice may use MHC or MUP characteristics or genome wide relatedness to recognise kin and choose a related partner (Manning et al. 1992; Green et al. 2015). Nevertheless, familiarity may also be important, since a laboratory study revealed that familiarity during juvenile development had a stronger influence on individual lifetime reproductive success than genetic relatedness (König 1994b). Therefore, we predicted d) that the other female came from the same litter or communal litter as the focal female (associated through juvenile familiarity) or she would be a similar age to the focal, and/or the other female was socially familiar (shares the same home area). Additionally,

given the importance of relatedness and a population's genetic structure in the evolution of cooperation (Hamilton 1964a; 1964b; Platt and Bever 2009), we analysed the genetic composition of the females' social environment in which they exhibited choice.

MATERIALS AND METHODS

Study Population

Data were collected from a free-living house mice population situated close to Zurich, Switzerland, from January 2008 until December 2009. This study period was longer than the average life expectancy of a mouse in the study population (average life expectancy: 196 d; Manser et al. 2011). The site was an old barn with a floor space of 72 m^2, which was divided into four equal sections by large plastic walls (holes in these walls enabled mice to access all sections). Each section contained ten nest boxes and numerous shelters that were distributed throughout. Wooden and plastic structures provided shelter and allowed the mice to form and defend territories. The barn, although closed to larger predators, was open to dispersal and immigration of mice, and to parasites and diseases (Dobay et al. 2015; Origgi et al. 2015). Food (50/50 mixture of oats and hamster food, Landi AG, Switzerland) and water was provided *ad libitum* in three feeding trays and four water dispensers per section.

All individuals of the population were captured every seven weeks. Over the two-year study period on average (mean ± SE); 104.5 ± 10 adult mice, 63.6 ± 9 subadults, and 37.6 ± 10 pups (when present, range: 0 – 112) were present during each event. Individuals weighing at least 18g were implanted with a subcutaneous transponder (RFID tag; Trovan-ID-100A implantable micro-transponder: 0.1g weight, 11.5mm length, 2.1mm diameter; implanter Trovan IID100E; Euro ID Identifikationssysteme GmbH & Co, Germany). Using a one-hand technique to restrain the mice the transponder was implanted with a sterile needle in the scruff of the neck, and a tissue sample was taken from the ear for genetic analysis (ear puncher, Napox KN-293: 1.5mm diameter). Once tagged, adults were individually identifiable allowing non-invasive monitoring of their position in the barn. Mice carrying RFID tags could either be identified with a hand-held transponder reader (during handling or when resting in nest boxes or shelters), or by an automatic antenna system that recorded the mice entering and leaving nest boxes (see below for a more detailed description). There have been no reported adverse effects of the transponders in this population or the literature. The Swiss Federal Law on Animal Protection recommends ear tissue samples for use as genetic tissue. Data collection was approved by the Veterinary Office Zurich, Switzerland (no. 215/2006).

More detailed information about the capture procedure, the barn set-up and the population can be found in König and Lindholm (2012); Auclair, König, Ferrari, et al. (2014); and König et al. (2015).

Reproduction

Reproduction was closely monitored in the nest boxes, which mice could access through a single plastic entrance tube. Experimenters were able to open the nest boxes at the top, allowing any litters born to be discovered and measured. Before opening a nest box we used a hand-held transponder reader to register the identity of all tagged mice inside. All shelters were also checked for tagged adults and litters, however, females rarely gave birth to pups outside of nest boxes (for all litters observed during the study period only 7% of litters

were found outside of nest boxes). Such nest checks were carried out during the day when mice were usually resting.

All nest boxes were searched for new litters every 8 - 12 days. These nest checks allowed us to find litters while they were still being nursed, and minimised disturbance of the nests by experimenters. As a consequence, however, litters were usually not found shortly after birth (28% of litters found between January 2008 and December 2009 were 1 - 3 days old, where day 1 is counted as day of birth). When a litter was found, age of the pups was determined using morphological indicators (skin pigmentation, teeth eruption, fur growth, and eye opening enabled age estimation of ± 1 day; König and Lindholm 2012; Auclair, König, Ferrari, et al. 2014). We further registered number of litters in the nest (1 litter = solitary; ≥ 2 litters = communal), and the litter size and age of each. When pups were estimated to be 13 days old, we took an ear tissue sample and morphological measurements. Day 13 was considered the closest age to weaning that we could safely handle and reliably locate pups, as pups open their eyes at day 14 and then attempt to escape capture (in terms of gaining independence from maternal nutrition, weaning starts at 17 days and ends at 21-23 days; König and Markl 1987).

Parentage Analysis

We took an ear tissue sample from every living pup when aged day 13, all handled adults and any corpses found. Following the procedure described in Auclair, König, Ferrari, et al. (2014), DNA was amplified at 25 microsatellite loci enabling parentage analysis assignment of mother and father for individuals, at a 95% confidence level using Cervus 3.0 (Marshall et al. 1998), success at assigning a mother to pups was between 87 – 88%.

Female Nest Box Use and Meetings

Every nest box had two antennas (NewBehavior AG, Zurich, Switzerland) fitted to the entrance tube allowing continuous monitoring of all tagged individuals coming and going (Fig. S1). Movement in and out of next boxes by a focal female was recorded by the antenna system and analysed for a tracking period of 30 days prior to the focal female giving birth. This time period was chosen as it included the gestation of the focal female and most of the gestation and initial lactation period of potential partners (house mice gestation: 19 - 21 days; König 2012). During this 30-day period the antenna system allowed us to calculate the number of nest boxes a focal mouse visited regularly. Females use a number of neighbouring nest boxes for resting and breeding (König and Lindholm 2012; König et al. 2015), and we determined an individual's home area from the nest boxes they entered. We also quantified the cumulated time a female spent in all nest boxes she entered as well as the frequency of these visits. Any nest box entered for less than 300 seconds within these 30 days was not considered as regularly entered and was excluded. Additionally, we determined all individuals a focal female met within this time period, the number of meetings they had, in which nest boxes they met and the total duration of these meetings (association time). This, therefore, provided a measure of recent familiarity between the focal female and each of her potential partners. For a detailed description of the antenna system and remote monitoring see König et al. (2015), and for an illustration of nest box stays and meetings see Figure S1.

In addition, we generated a null model of expectation to test female choice against random expectation. This null model calculated the probability of a litter being randomly chosen given the proportion of nest boxes that contained a litter during the 16 days before a female gave birth (nest boxes considered were only those used regularly by the female of

interest). Proportion of occupied nest boxes was determined using the number of nest boxes that had at least one litter born into them, and dividing by the total number of nest boxes a female used (for example, a female used 5 nest boxes, and in 2 of these boxes a litter had recently been born, meaning 40% of her nest boxes were occupied). If two or more litters were born in the same nest box, this was classified as 1 occupied site. We tested our observed data against the random expectation that the probability of joining another litter was equal to the proportion of nesting sites occupied. This model allowed us to test whether communal nursing was an artefact of communal living.

Female Communal Nursing Options

A litter is considered communal when two or more females pool their litters in the same nest. Once litters are pooled a female with pups already present in the nest is unable to discriminate between own and non-offspring (Chantrey and Jenkins 1982; König 1989; Manning et al. 1995; Ferrari et al. 2016). Thus for the purpose of this study we determined the decision to nurse communally as being made by the pregnant female who was about to give birth (hereafter: focal female) and not by those that had already given birth (meaning in the event a female chose to nurse solitarily, her litter could later have been joined and become communal). Any female (hereafter: option female) that gave birth within the 16 days before a focal female gave birth was classified as a potential communal nursing option. According to Weidt et al. (2014), females only communally nurse when they share the same social group, here based on shared use of nest boxes. We also never found that females communally nursed a litter with a female that did not have use of overlapping nest boxes. Thus, available options for each focal female were considered to be those litters born to females in the nest boxes regularly entered by the focal female (determined by the antenna system, as explained above). We chose sixteen days before the focal female gave birth as the limit for communal nursing options, as 16 day old pups are still nursing, but weaning starts at 17 days of age when pups begin to eat solid food (König and Markl 1987). Such criteria has been used in several other studies of communal nursing (König 1994a; Weidt et al. 2014; Ferrari et al. 2016). Our intention was to gain information from partner choice in the presence of at least one available option. Therefore, all situations in which a female had no available option to communally nurse at the time of giving birth, in her social environment, were excluded from our analysis.

For each option available we compiled the following data on the focal and option females, as well as their litters: association time, pairwise relatedness and age difference between the focal and the option female, age difference between the focal and option litter, and number of nest boxes the focal and option female shared. Using this dataset we determined what factors influenced a focal female's decision to communally nurse. We had two categories of option females (Fig. 1): (1) Chosen partner (C), the option female that the focal female chose as a communal nursing partner; (2) Non-chosen partner (NC), the option female(s) that the focal did not choose to form a communal litter with; these also include the option females from the cases where the focal female chose to nurse solitarily (F2 in Fig. 1).

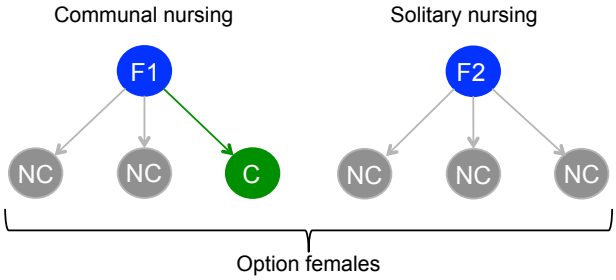

Figure 1. An illustration of the option female categories. Blue circles illustrate the focal female, either in a scenario where she opted to join another litter and to nurse communally (F1) or where she opted to have a nest with only her litter and nurse solitarily (F2). All option females are shown as either non-chosen partners (NC, grey circles) or a chosen partner (C, green circle).

Litters were initially considered solitary when all pups morphologically looked to be at the same stage of development. In some cases later genetic analysis revealed "cryptic" communal litters in which two females had given birth to litters on the same day in the same nest box. In the event that a cryptic communal litter was found with no other litter, we could not be certain which female gave birth first. Therefore, we randomly chose one of the females to be the focal female, as in such a case all attributes between the two females would be equal, for example pairwise relatedness, association time and age differences. In two cases a focal female joined an existing cryptic communal litter. In this scenario it was not clear which option female had been chosen, therefore, we took the average value for each characteristic.

Another predictor of choice that we considered was juvenile familiarity between a focal and an option female, which was determined when both females were raised in the same litter (having the same mother and found in the same nest on the same day), or in the same communal litter, with a maximum age difference of 16 days. We were further interested to test the effect of litter size on the focal female's decision to choose one option over another. However, we could not be certain that our measure of litter size for each litter was an accurate representation of birth litter size. We were only able to take the number of pups that were present when we found the litter for the first time. A more accurate measure of litter size would have required checking nest boxes daily, which would have increased disturbance and in such situations females often relocate litters elsewhere (pers. obs.). Furthermore, pregnant female house mice are known to kill pups already present in the nest before giving birth themselves (McCarthy et al. 1986; König 1994b; 1994a; Ferrari et al. 2016), which could reduce observed litter sizes.

To consider an effect of population density on the decision to communally nurse in our study, we took the adult population density calculated from the population-monitoring day closest to the birth date of the focal female's litter. To assess seasonal effects on reproduction (see König and Lindholm 2012) we included in our analyses the season the litter was born (Summer = March to August; and Winter = September to February).

To analyse choice of communal nursing partner we were specifically interested in the factors of an option female and her litter that determine whether a focal female will form a communal litter with her or not. Therefore, to analyse such data we chose focal females for which we had complete information about all potential option females at that given decision from our full dataset, which reduced the data to 74 events. This reduction in data occurred because of missing parentage information of option females, and in some cases the potential option litters were not found again at day 13 (due to pup mortality, that was likely due to infanticide; Auclair, König and Lindholm 2014), therefore no genetic sample could be taken from the pups and consequently no mother could be assigned. Of these 74 we focused on the 34

occasions (N = 28 individual females) when a focal female chose to nest communally. We used the remaining 40 events to make comparisons with the communal options.

Pairwise Relatedness Measures and Spatial Genetic Structure

We compared how genetically similar two individuals were to each other at 25 microsatellite loci to the average similarity between dyads of the year the focal female's litter was born (either 2008 or 2009). To choose an appropriate estimator we took from our pedigree 50 full sibling and 50 parent-offspring dyads of expected relatedness r = 0.5, 50 half sibling dyads (expected r = 0.25), and 50 dyads of unrelated individuals (expected r = 0; living contemporaneously and not sharing a grandparent). For all these dyads we estimated pairwise relatedness values using seven different estimates for r and correlated them against the pedigree r values as implemented in Coancestry (Wang 2011). From this we determined the Wang estimate (Wang 2002) to have the highest correlation (R = 0.80) between pedigree r and estimated r, and therefore used this to determine pairwise relatedness in the current study.

We further assessed the spatial genetic structure of females in the entire barn during the years monitored using GenAIEx 6.5 (Peakall and Smouse 2012). This spatial genetic autocorrelation analysis allowed comparison of genetic similarity between female mice depending on their location in the barn during nest checks. We calculated a genetic distance matrix using microsatellite genotypes. A spatial location was assigned to each female based on the nest box where she was detected, at the time of a nest check (when each nest box was scanned by a handheld reader). To reduce autocorrelation, the first nest check in each month was used (N = 23 nest checks). Spatial genetic autocorrelations were computed between a focal female's genotype and the genotypes of all other females at the same location (radius of 0; starting point), and between the focal female and all females recorded within increasing concentric circles of 1 m radius from the starting point. Since neighbouring nest boxes are generally located within 1 m of each other, a radius of 1 m typically included 2 - 3 nest boxes. This was repeated for all females and significance was determined by random permutations.

Statistical Analysis

Statistical analyses were carried out using R version 3.1.3 (R Core Team 2015). Generalized linear mixed models (GLMM) with binomial error distribution and logit link function were used to allow for the inclusion of random effects and dependencies in the data. All mixed models were performed using the R package 'lme4' (Bates et al. 2014), and fulfilment of model assumptions were inspected visually from the model diagnostics (Zuur et al. 2009). Some explanatory variables were on varying scales, therefore, to improve interpretability of parameter estimates (Schielzeth 2010) we centred and scaled the continuous explanatory variables in the appropriate models (association time, pairwise relatedness values, age difference between the females, partner age and focal female age).

We analysed the probability of a focal female choosing to communally nurse using a binomial GLMM (option taken = 1, option not taken = 0). We specified a full model which included the following fixed effects: number of options available, number of nest boxes entered, age of the focal female, experience of focal female (whether the focal female had a litter before), adult population density, and season. Female identity was included as a random effect.

We further assessed the probability of an option female being chosen using a binomial GLMM (chosen female = 1, non-chosen female = 0). The fixed effects in our full model included: age of the option females pups on the day the focal female's pups were born, litter

size difference (absolute), number of nest boxes shared, age difference between the focal and option female, age of option female, pairwise relatedness, association time with the option female, and whether the option litter was solitary. Event ID (a unique number given to each decision), focal female identity and option female identity were included as random effects. We were unable to specifically test juvenile familiarity due to incomplete data; we therefore looked at cases where the information was available to assess its occurrence, and therefore used age difference between the females as a proxy.

We carried out initial model selection on both GLMMs to determine whether the interaction terms were important by using the model selection function in the MuMIn package (Bartoń 2015). Our models were compared to all possible combinations of that model containing the same or fewer interaction terms, and to a model containing no interactions, and all fixed effects were kept in the model. Models were ranked by corrected Akaike information criteria (AICc), whereby the model with the lowest AICc value was chosen to be the most adequate model. In the event that two or more models fell within 2 delta AICc of each other, we then chose the model with the lowest degrees of freedom. In both cases the most adequate model was the model containing no interaction terms, and therefore all interactions were considered non-significant $P > 0.05$. To determine the significance of each fixed effect, we compared a model with the fixed effect of interest removed to the model containing all fixed effects, using likelihood ratio tests (Crawley 2007; Forstmeier and Schielzeth 2011). All random effects were kept in the model and variance components were estimated using maximum likelihood methods.

To test the difference between the pairwise relatedness of the focal female to the option females in the communal nursing scenario against those of the focal female to the option female(s) in the solitary nursing scenario, we used a linear mixed model (LMM), with Event ID, focal female identity and option female identity included as random effects. To assess whether age of pups had an effect on the association time between the focal and option female we used a LMM with age of pups included as an explanatory variable, and the same random effects as above. To determine whether communal non-chosen options and the solitary non-chosen options differed with regard to age of pups, we used a Wilcoxon-Mann-Whitney rank sum test, as in this case the data were not normally distributed (tested using a quantile-quantile plot; Crawley 2007).

To assess the prediction that communal nursing was an artefact of communal living, we generated a null model. Using a non-linear least squares model (NLS), we tested whether our observed data differed significantly from a random expectation, whereby the probability of choosing to nurse communally was equal to the proportion of occupied nesting sites.

RESULTS

During the two-year study period we collated information on 314 litters producing 1,432 pups. In 276 cases ($N = 127$ individual females) the focal female had at least one option to nest communally. In the remaining 38 cases no other female gave birth in the previous 16 days, meaning they had no option but to nest solitarily (these were excluded as focal females). The number of females considered as an option (altogether $N = 128$ individual females) ranged from 1 to 15 within a female's home area (Fig. 2b; determined according to nest boxes visited regularly). Females chose to nest communally in 106 cases (38.3%; $N = 77$ individual females) and solitarily in 170 cases (61.6%; $N = 98$ individual females); 48 females used both nursing strategies during the study period.

Table 1. Mean ± standard error of mean (SE) for attributes of the option females under the different scenarios, whether the partner was chosen as a communal nursing partner or not.

	Focal chose CN		Focal chose SN
Attributes of the option females	Chosen	Non-chosen	Non-chosen
Age of pups (d)	2.7 ± 0.5	8.0 ± 0.7	6.6 ± 0.6
Litter size difference	3.7 ± 0.7	2.9 ± 0.5	2.2 ± 0.1
Number of boxes shared	2.7 ± 0.2	2.7 ± 0.2	2.3 ± 0.2
Age difference between females (d)	141.8 ± 24.4	190.7 ± 28.6	136.5 ± 16.4
Age of option female	297.2 ± 23.8	301.0 ± 30.8	336.3 ± 16.6
Pairwise relatedness	0.30 ± 0.04	0.26 ± 0.03	0.19 ± 0.04
Association time (min)	4303.1 ± 720.3	2098.3 ± 402.4	2458.0 ± 368.9

CN, communal nursing; SN, solitary nursing; Litter size difference, the absolute difference between focal female litter size and the option female's litter size. Age difference between females is given as absolute days, and is calculated from the date the focal female gave birth.

Communal Nursing Decisions

Focal females were significantly more likely to nurse their litters communally with increasing number of available options in their home area (Fig. 2a, Table 2). Adult population density (taken from the closet population-monitoring event) did not significantly affect the decision to nurse communally (Table 2). More litters were born in summer (March to August; N = 228; communal = 90, solitary = 138) than in winter (September to February; N = 48; communal = 15, solitary = 33), but there was no significant influence of season. Unsurprisingly, the number of options available to a female increased with number of nest boxes used by the focal female, but this had no significant effect on her decision (Table 2). Females sometimes chose to nurse solitarily even when there were up to 13 potential partners available. Additionally, there was no significant effect of reproductive experience of the focal female (whether she had a litter before; Table 2) on her decision to nurse communally. In addition, females on average used 5.20 ± 0.10 (mean ± SE) nest boxes and interacted with 8.69 ± 0.24 (mean ± SE) females (includes all female interaction partners of the focal female during the 30-days prior to birth, reproducing and non-reproducing).

Table 2. Results from the generalised linear mixed models explaining the decision to nurse communally and choice of communal nursing partner.

Response variable	Explanatory variables	Estimate	CI	Likelihood ratio test (χ^2)	*P*
Option taken	**Number of options available**	**0.14**	**0.01, 0.26**	**4.89**	**0.027**
	Number of nest boxes entered	-0.07	-0.24, 0.10	0.66	0.418
	Age of the focal female	0.10	-0.21, 0.41	0.42	0.518
	Experience of focal female	-0.54	-1.20, 0.12	2.59	0.108
	Adult population density	0.28	-0.43, 0.99	0.58	0.447
	Season	-0.26	-0.97, 0.46	0.51	0.477
Partner chosen	**Age of option pups**	**-0.44**	**-0.66, 0.22**	**26.92**	**<0.001**
	Litter size difference	-0.32	-1.05, 0.40	0.75	0.387
	Number of nest boxes shared	-0.19	-0.90, 0.53	0.27	0.606
	Age difference between the females	-0.57	-1.23, 0.09	2.90	0.088
	Age of option female	-0.20	-0.90, 0.49	0.33	0.565
	Pairwise relatedness	0.12	-0.56, 0.80	0.11	0.738
	Association time	**0.95**	**0.01, 1.89**	**4.39**	**0.036**
	Was option litter solitary?	-0.24	-1.72, 1.23	0.10	0.749

Explanations: Experience of focal female = whether a focal female had a litter previously or not (whereby 1 = yes and 0 = no, first litter); Age of the focal female, age of potential partner and age difference (absolute) between the females = calculated as the age the mouse would have been at the time the focal female gave birth to her litter; Option taken = whether the focal female chose to form a communal litter (1) or whether she decided to nurse solitarily (0). Partner chosen = whether the option female was chosen (1) or not (0). Significant factors ($P < 0.05$) are highlighted in bold.

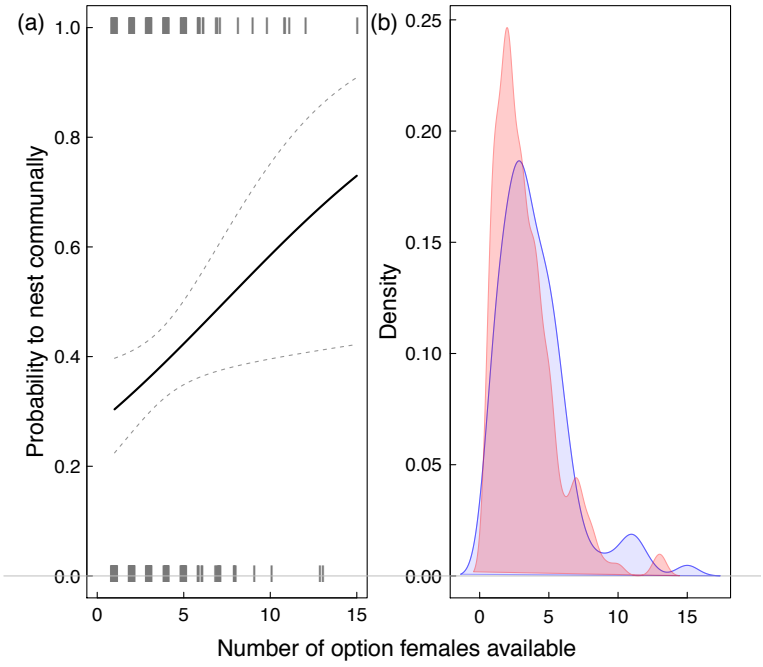

Figure 2. a) Probability of a focal female choosing to nurse communally (score = 1) vs. solitarily (score = 0), here shown against the number of potential communal nursing partners available at a given decision. Tick marks demonstrate the variability in the number of options available. **b)** Density plots of the number of available options for the females that chose communal (blue) and the females that chose solitary (red).

Results from the null model indicated that our data was significantly different from a null expectation (NLS: t = 2.57, $P = 0.014$, null expectation: $\boldsymbol{a} = 1$, observed data: $\boldsymbol{a} = 1.30$, Fig. 3), which suggested females chose to nurse communally less frequently than under random expectation.

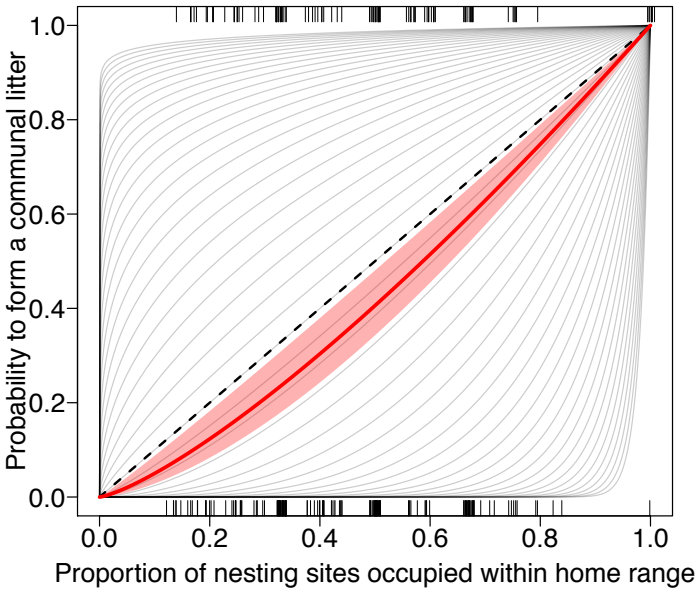

Figure 3. Null model of expectation: dashed line represents $\boldsymbol{a} = 1$ suggesting choice of partner was equal to the proportion of occupied nesting sites, if $\boldsymbol{a} < 1$ females choose communal (1) more often than random expectation and, if $\boldsymbol{a} > 1$ females choose solitary (0) more often than random, potential values for $\boldsymbol{a}$ are represented by the light grey lines. For the raw data we find a value of $\boldsymbol{a} = 1.3$, here represented by the red line (± 95% CI polygon). Tick marks represent the variability in the proportion of occupied sites.

Choice of Communal Nursing Partner

Neither litter size difference, nor pairwise relatedness significantly affected the decision to choose one partner over another (Table 1, 2). However, there was a significant difference between relatedness of all option females in the communal scenario (overall mean of all option females when a focal chose communal nursing: r = 0.235 ± 0.02) and those of all the option females in the solitary scenario (mean: r = 0.192 ± 0.02; $\chi_1^2 = 4.57$, $P = 0.033$). Females thus chose to nurse communally when her available options were generally more closely related to her. We investigated the genetic spatial structure of the females for the two years that the study population was monitored, and found that females had significant positive genetic correlations with other females found in the same nest box (0 cm) and up to 400 cm from that nest box (significant positive genetic spatial structure was observed at: 0, 100, 200, 300 and 400 cm, $P < 0.001$, Fig 4). As distance from the central point increased, these correlations declined to zero and below, implying that female mice were found close to genetically similar individuals.

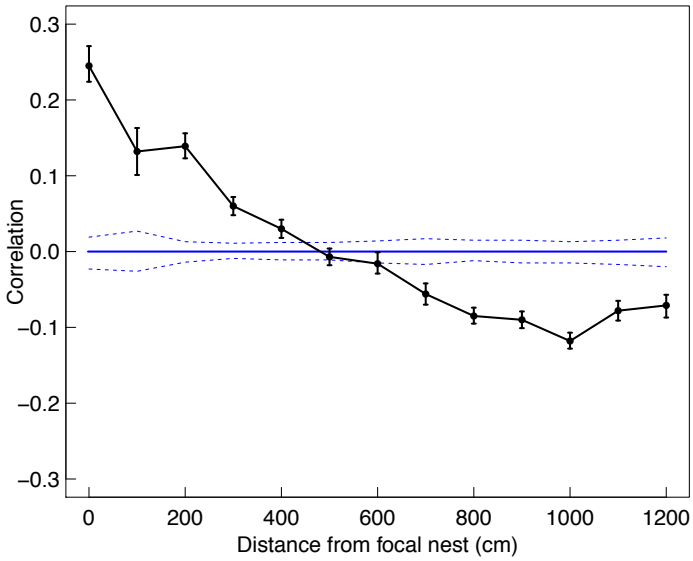

Figure 4. A plot illustrating the genetic spatial structure analysis for all females in the study population, during 2008 and 2009. Estimates and 95% CI are shown per distance class. Blue line represents the average (zero) with a 95% CI, illustrating the null hypothesis of no spatial structure. All confidence intervals were obtained by bootstrapping.

We found that the likelihood of an option female being chosen was significantly increased when her pups were young (Table 1, 2). 82% of the focal females joined a litter that was maximally 5 days old, and 72% of the females chose a litter that was younger than the average of her other available option litters (all females that chose to nurse communally from the full data set, N = 105). To test if age of pups influenced a focal female's decision to solitarily nurse, we compared the age of pups in the option litters available to a focal female that chose solitary nursing against the age of pups in the non-chosen litters of the communal nursing females. We found no significant difference (Wilcoxon test; W = 1048, P = 0.146), suggesting that the availability of a young litter played an important role in a focal female's decision.

For juvenile familiarity, 27 option females (18.8%; 27 of 148 option females) were of a similar age to the focal female (differing by a maximum of 16 days in age). Twelve of these were females born in the same litter (siblings) or in the same communal litter as the focal female. Of the females that chose to nurse communally, only 6 focals had the option to choose a litter sibling, and in only 2 cases they choose her as a partner, one female chose a maternal sibling and the other a full sibling. Therefore, only a few focal females (12.6%) in this data set had the option to raise a communal litter with a partner that was from the same litter, and therefore familiar by juvenile association. Furthermore, there was also no significant effect of age difference between the focal and the option female (Fig. S2), or option female's age on the focal females decision (Table 1, 2).

However, we found that focal females spent significantly more time with the chosen partner in the month before birth than with her other option females (Table 1, 2), suggesting the chosen partner was more familiar. There was no significant effect of age of pups on the time females spent together in the month before the focal female gave birth (χ_1^2 = 3.21, P = 0.073), suggesting that time spent with the chosen females was not an artefact of her having young pups.

DISCUSSION

Our results indicated that females were 'choosy' in their decision to nurse communally, whereby they did not always choose to communally nurse when they had the opportunity. Females shared nest boxes and regularly met with on average only 8 – 9 females (including non-breeding females) in their overlapping home area, and thus seemed to establish fairly closed social groups. Within such groups, females generally chose to nurse their litters solitarily, a decision that was more likely than random expectation. They did so even when there were up to thirteen potential communal nursing partners available in their home area. Adult population density did not affect a female's probability to choose communal nursing. Hence, these observations do not support the hypothesis that communal nursing was a by-product of sharing the same social environment or nesting sites. On the contrary, they reinforce earlier results observed by Weidt et al. (2014), who studied the same population 5 years earlier when the population size was much lower (maximal density: 0.94 adults / m^2), and comprised only 36% of the maximum population density analysed in this study (minimum: 0.72 adults / m^2; maximum: 2.61 adults / m^2).

The probability that a focal female chose communal nursing increased with the number of potential partners, suggesting the probability of a preferred communal nursing partner being available increased when more partners were available. This supports our initial expectation that choice of partner is an important aspect in female cooperation when rearing litters together. Most interestingly, choice was exhibited in a social environment that was composed of genetically similar individuals (a female's shared nesting sites or home area consisted of mainly relatives), and within such social groups females chose partners that were familiar and had recently given birth.

Communal Nursing Decisions

We predicted that females would more often choose an existing solitary litter due to individual lifetime reproductive success decreasing below solitary nursing females when three or more females pool their litters (König 2006). However, we found no effect of litter type on the decision to nurse communally. Females did not differentiate between joining an existing solitary or communal litter, and we observed from the null model that females tended towards solitary nursing. This suggests that the decision to nurse communally was dependent on the availability of a preferred partner at that given time. Contrary to our prediction, litter size did not play a significant role in explaining choice of communal nursing partner. Empirical studies have shown that females avoid communal nursing when litter size at birth is uneven (Ferrari et al. 2016), however, in a free-living environment litter size may not be an important cue in communal nursing decisions. We must also consider that pregnant females are known to manipulate a partner's litter by killing one or several of her pups before giving birth herself (McCarthy et al. 1986; König 1994a; 1994b; Ferrari et al. 2016), which could result in the observed litter sizes being reduced prior to them being found, and could potentially have hidden an effect.

According to kin selection theory relatedness is required for the evolution of costly cooperation (Hamilton 1964a; 1964b; Taylor 1992; West et al. 2001; Queller 2011), and is assumed to explain cooperative behaviour not only in vertebrates (birds: Cockburn 1998; mammals: Clutton-Brock 2002), but also in social insects (Bourke and Franks 1995; Queller and Strassmann 1998), and bacteria (West et al. 2007). We found no effect of pairwise relatedness on the decision to nurse with one partner over another. However, females may have

been less inclined to fine-tune their discrimination (Hamilton 1964a; 1964b; Mitteldorf and Wilson 2000; Perrin and Lehmann 2001) given that the females in their home area were genetically similar to them. This would mean investment into another female's offspring during communal nursing could be explained by the indirect fitness benefits gained. Exploitation costs are also more often tolerated when cooperating with a relative (West et al. 2002; Mathot and Giraldeau 2010), which is supported by our observation that focal females chose to communally nurse more often when the general pairwise relatedness of their options was higher. Therefore, by preferentially grouping with close relatives females can minimise maternal investment in unrelated young (Manning et al. 1992). Seeing as a female's home area consisted of genetically similar females, familiarity, or a prior association, between females may also play a role in communal nursing decisions.

Juvenile familiarity, when unrelated females are raised together in the same nest, was shown to have major importance in laboratory studies with wild-bred house mice (König 1994b). Females had increased offspring survival with a familiar unrelated partner they grew up with over an unfamiliar sibling. In our study, we observed that females very rarely had the opportunity to communally nurse with a litter sibling, or with a mother or daughter. Such lack of opportunities can be explained by the low average life expectancies in house mice (Manser et al. 2011), high pup mortality (Auclair, König, and Lindholm 2014), reproductive skew (König and Lindholm 2012), or the possibility of dispersal from the study population. Even if a female shared a home area with a litter sibling, we predict that the chance of both females having litters within such a short period of time is likely to be low. Here, we can rule out juvenile familiarity as a decisive factor, since only 6% of focal females chose a sister from her birth litter. We further found females did not discriminate their choice by age of the option female, if juvenile familiarity was important we would have expected females to prefer those that were young and/or similar in age to them.

However, we did find that females choose a partner who they had associated with most often during the month before giving birth. This suggested a preference for a partner who was more familiar (a female she had spent more time with in nest boxes) during her pregnancy, or who was at a similar stage of pregnancy to her. However, this may have been confounded by the changes in activity patterns of lactating and pregnant females, as lactating females were shown to modify their daily activity pattern by alternating between nursing and being out of the nest (see Auclair, König, Ferrari, et al. 2014). This could mean that focal females spent longer periods of time with an option female in the same nest boxes when both were simultaneously pregnant. Therefore, prior knowledge of their potential partner or the partner's stage of pregnancy may have been a contributing factor in communal nursing decisions. However, familiarity can be used as a mechanism to recognise kin via prior association or phenotype matching, where another's phenotype is matched to self or a known relative (Hauber and Sherman 2001). In a free-living population, however, it is difficult to exclude phenotype matching as a template for familiarity (Pfefferle et al. 2016). Female house mice are considered the philopatric sex (Gerlach 1990; Dobson et al. 2000; Rusu and Krackow 2004), suggesting that females living in the same home area would likely be familiar by default. Therefore, to better understand whether familiarity is a factor behind communal nursing decisions future studies could use semi-natural enclosures, where the genetic background of groups of females can be pre-determined.

Choice of communal nursing partner may also have occurred under a hierarchy of cues as suggested for mate choice (Mays and Hill 2004). Female mice demonstrated complexity in their decision-making, whereby their choice was affected by the variance in characteristics of the males (Roberts and Gosling 2003). In their study, MHC dissimilarity predicted mate choice

only when the variability in male scent marking rates (high scent marking indicates a higher dominance rank) was low (Roberts and Gosling 2003). Females in the context of communal nursing decisions may also adopt such a strategy by using alternative cues to choose a partner from a group of females when the variance in pairwise relatedness or familiarity is low. Furthermore, it is possible that mice assess relatedness at specific loci instead of genome wide, particularly at the MHC and MUP genes (Manning et al. 1992; Roberts and Gosling 2003; Green et al. 2015). Therefore, in our study population it is possible that overall genetic relatedness is a less important cue than MHC or MUP similarity; future studies could investigate such differences and their influence on communal nursing decisions.

Effect of Communal Litter Age Disparity on Choice

We found that focal females preferred to join another female when her partner's pups were young, with 82% of these being less than or equal to five days old. This finding compliments those of Manning et al. (1995), who found females were more likely to choose communal litters with pups younger than the average of the other available litters. Females may have chosen a younger litter to prolong the period of time that the partner female was unable to discriminate between offspring, and increase the chance both litters would be nursed equally. Some studies have shown females are more likely to discriminate between offspring in a communal litter when the age disparity between them is larger (Manning et al. 1995; Hayes 2000; Schmidt et al. 2015; Tučková et al. 2016), however in house mice it is believed that females cannot discriminate between own and non-offspring before the onset of weaning (Chantrey and Jenkins 1982; König 1989; Manning et al. 1995; Ferrari et al. 2016), or at least have a limited ability to do so (Hager and Johnstone 2005). However, effective nest defence is likely highest in the days following birth, and postpartum aggression in females during this time was shown to be highest during the first 3 days, in particular towards unfamiliar intruders (Svare and Gandelman 1973). Therefore, joining a familiar female with younger pups could ensure higher nest defence, reducing the chance of infanticide by intruders. Furthermore, although inter-litter competition has only been speculated in house mice (Hayes 2000; Hager and Johnstone 2007), competition between pups should be less costly when the age between the two litters is smaller (Hayes 2000). Females may have been attempting to avoid inter-litter competition, and therefore, promoted own offspring survival by choosing litters for which between litter competition would be low. To better understand the reasons behind choice of a litter with young pups, and disentangle other factors such as familiarity or relatedness, a different data set or use of empirical studies where factors can more easily be controlled and modified would be required. Furthermore, future studies could explore the implications of these decisions and the fitness consequences they may have.

Conclusions

Our study suggests that the decision to communally nurse in a free-living house mice population occurred less often than expected by chance, but with increasing available options there was a higher probability of occurrence. Through analysis of the females' spatial genetic structure within the study population, we determined that females shared their social environment with genetically similar individuals. Such results indicate female's shared their home area with related females that could have favoured the evolution of cooperation through communal nursing. It may even suggest that due to the low variance in pairwise relatedness a female's decision may then depend on other factors from the pool of options available. Taken

together, our findings show that communal nursing is not a by-product of sharing the same nesting sites, and that female house mice have the capacity to choose a communal nursing partner from a social group of genetically similar females, and in doing so preferred those that are familiar and have young pups.

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SUPPLEMENTARY MATERIAL

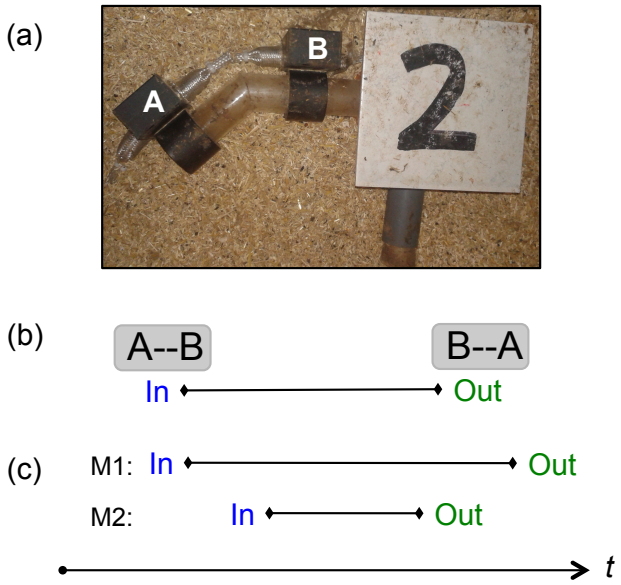

Figure S1. An illustration of nest box stays and meetings. **(a)** Birds eye view of a nest box in the barn (here box 2), the white square indicates the nest box lid (ceramic tile), the two black boxes indicated by the letters A and B are the outer (A) and inner (B) antenna, which are attached to a clear acrylic entrance tube (this photograph illustrates our new antenna system (AniLoc system, FBI Science GmbH, Germany), however, the position of the antennas and method of reading transponders was the same as the previous system, NewBehavior AG, Zurich, Switzerland). **(b)** Two antennas are required in order to distinguish between a mouse entering and leaving a box, which allowed us to determine a nest box stay. When a mouse entered a box it was read first by antenna A followed shortly after by antenna B, and vice versa when it left the box (each nest box had two unique identifiers for the antennas). The time (s) between the 'in' and 'out' readings was classified as a nest box stay; **(c)** Illustrates how nest box meetings between different individuals was monitored, classified by the time (s) in which 2 mice (mouse 1 (M1) and mouse 2 (M2)) overlap inside the nest box, we can also calculate the number of times they meet (counts).

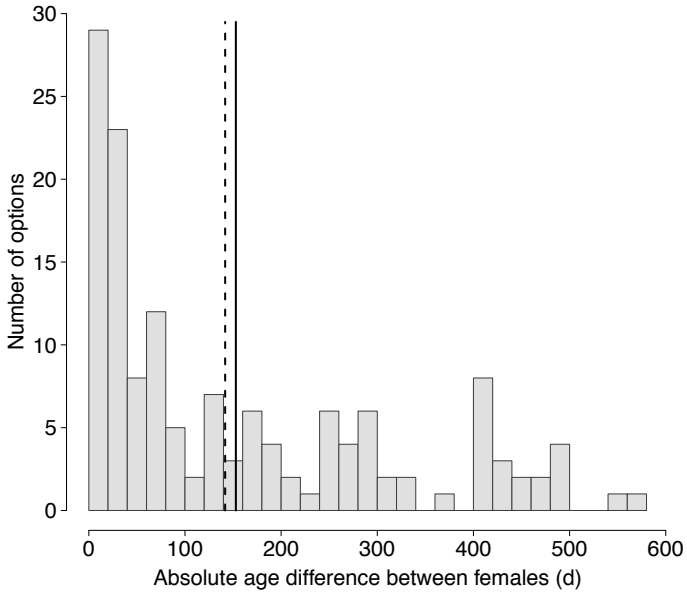

Figure S2. Plot to illustrate the distribution of absolute age difference (in days) between focal females and all available options (irrespective of whether an option was chosen or not). The solid line indicates the mean age difference for all options (152.9 d), and the dashed line represents the mean age difference for the chosen option females only (141.8 d). This figure demonstrates that although right-skewed towards smaller age differences the majority of options had an age difference of more than 20 d, and age difference varied.

CHAPTER 2

Same-sex Social Preferences Among Free-Living Female House Mice

To be submitted

Same-sex Social Preferences Among Free-Living Female House Mice

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ABSTRACT

Sociality commonly results in fitness benefits whereby same-sex individuals form strong and stable bonds with one or several of their conspecifics. The benefits associated with these social partnerships include increased offspring survival, increased longevity, reduced stress and better group cohesion. Such positive links between social preferences and fitness advantages have been observed in a variety of species. However, the majority of studies investigating preferences among conspecifics and the stability of these preferences over time have focused on primates. In house mice, experimental laboratory studies with wild-derived mice revealed that females form preferences for specific partners and associate more often with kin. Therefore, we aimed to verify the existence of such preferences in a free-living population of house mice analysed over a period of two years, and determine if they were stable over time. By calculating sociality scores we were able to determine differences in the extent of associations between females. We found that within a focal female's social environment she interacted with other females to differing degrees, and the strength of these dyadic associations significantly increased with increasing pairwise relatedness. We further determined that preferences were stable over time and some female dyads occurred over consecutive months. We also demonstrated that, within a specified time period, reproduction (as reflected by the number of litters raised) increased with decreasing number of females in a social group. In accordance with reproductive seasonality of the study population, females reproduced less when reaching reproductive age in winter. This study quantified social interactions among female house mice in a free-living context, and provided evidence that they form affiliative, stable relationships preferentially with relatives over several months.

INTRODUCTION

Group living has been studied in a range of species (Holekamp et al. 1997; Sapolsky et al. 1997; Silk. et al. 2006a; 2006b; Barocas et al. 2011; Kerth et al. 2011; Perony et al. 2012), and commonly occurs when the costs of interacting with conspecifics are offset by the fitness benefits gained (Krause and Ruxton 2002; Silk 2007). The benefits acquired from living in a social group can be influenced by different factors that are dependent on the species of interest and their habitat, including aspects such as group size and composition, as well as strength and stability of social preferences (reviewed in Silk 2007). Female baboons, *Papio cynocephalus*, that lived in small social groups consisting of close kin had more stable social relationships than females living in larger groups with fewer close kin, and strong social preferences between females were often maintained for several years (Silk et al. 2006a; 2006b; 2010a; 2012). Male chimpanzees, *Pan troglodytes*, demonstrated strong and stable dyadic associations that were important in dominance strategies (Gilby and Wrangham 2008), and spotted hyenas, *Crocuta crocuta*, sustained strong social relationships with kin year round despite resource limitation (Holekamp et al. 1997). Within the context of social relationships, individuals that form alliances or bonds with a same-sex partner may benefit from increased offspring survival, longevity and increased group cohesion (König 1994a; Silk et al. 2003; Yee et al. 2008; Seyfarth and Cheney 2012). Strong and stable bonds with female conspecifics increased life span in chacma baboons, *Papio ursinus* (Silk et al. 2010a), and in savannah baboons female sociality was positively correlated with offspring survival (Silk et al. 2003). Furthermore, evidence linking social preferences with reproductive success has been observed in bottlenose dolphins, *Tursiops sp.* (Frére et al. 2010), female horses, *Equus equus* (Cameron et al. 2009), and female house mice, *Mus musculus domesticus* (Weidt et al. 2008). However, studies investigating the strength and stability of preferences between females have primarily focused on primates (Silk et al. 2013), and were descriptive. On the other hand, in house mice laboratory experiments revealed the presence of individualised preferences among sexually mature female house mice, and that those preferences led to improved individual lifetime reproductive success (Weidt et al. 2014). Based on such results we here intended to verify the existence of preferences among females from a long-term study on wild house mice.

House mice are a group living, social rodent. Groups are usually composed of a territorial male, a few subordinate males and a number of breeding and non-breeding females (Anderson and Hill 1965; Lidicker 1976; MacKintosh 1981; Weidt et al. 2008). Female mice are frequently considered philopatric and remain in their natal territory, but can when required successfully disperse and reproduce in new territories, where they will interact with unfamiliar individuals (Anderson and Hill 1965; Baker 1981; Gerlach 1990; Weidt et al. 2008). Reproduction is unevenly distributed among females leading to competition over breeding opportunities and weaning of offspring (König 1994a; 1994b). Therefore, a predominant focus of laboratory studies has been the cooperative nature of female house mice, more specifically their use of communal nursing. Here two reproducing females pool their litters in the same nest and nurse each other's pups indiscriminately (König and Markl 1987; König 1994a; Hayes 2000; Ferrari et al. 2015). In doing so females profit from fitness advantages, such as increased lifetime reproductive success in comparison to nursing pups alone (solitary nursing), and seem to obtain the highest success when nursing with a related, familiar or preferred female partner (König 1994a; 1994b; Weidt et al. 2008).

In order to accomplish cooperation that benefits both females, it is believed that choice of communal nursing partner is important (Weidt et al. 2008; 2014; Harrison et al. in prep), especially as empirical evidence has shown a potential for exploitation (Ferrari et al. 2015;

2016). In laboratory enclosures, female house mice were shown to form preferences towards other females with whom they had greater reproductive success than when paired with non-preferred partners (Weidt et al. 2008). Additionally, Perony et al. (2012) demonstrated that mice in a free-living population regularly met with members of their social group, and recent evidence determined that females shared nesting sites in their home area with related conspecifics (König et al. 2015; Harrison et al. in prep). Also when females were presented with a number of potential communal nursing partners they preferred those that they had spent more time with in the month before birth (Harrison et al. in prep). Given these findings, and that female's nurse with different, even multiple partners during their reproductive life span (Auclair et al. 2014b; average females nursing communally together at one time was 2.9), it can be assumed that choice of communal nursing partner is important, and that females potentially form social preferences with numerous females. However, despite evidence that females form social preferences in laboratory enclosures (Weidt et al. 2008), surprisingly little is known about the social interactions of female mice in a free-living context, and whether they do form social preferences.

Following techniques obtained from a range of primate studies (Cheney 1992; Silk et al. 2010a; 2010b; 2013; Cheney et al. 2012), we used a free-living population of house mice where interactions could be monitored remotely through the use of a tracking system. In their natural environment, house mice depend on places or nests that provide protection from predators, and a suitable microclimate to allow for survival and successful reproduction (Crowcroft and Rowe 1958; Hurst 1987; König and Lindholm 2012). In social species, the frequency and duration of meetings in the same nest or shelter is considered to reflect a type of spatial association (Kerth et al. 2011). Assuming that regular and long meetings between individuals in the same nest reveal a socio-positive relationship (at least not predominately agonistic), overlapping use of nest boxes can be used to assign social group membership (for a detailed discussion see König et al. 2015). For the purpose of the current study, we extracted information on female social interactions from use of nesting sites, and calculated the time a female spent with each female present in their social group over a specified time period. We then determined whether females had particular partners they interacted with more often, and examined whether these preferences were stable over time. We further determined whether group size, relatedness or age difference to a group member affected these preferences, and how they influenced reproduction. Based on results previously gained in the laboratory, we predicted that free-living adult female house mice exhibit social preferences for certain female conspecifics, revealed by affiliative social interactions. We predict that these social relationships are stable over time and improve reproduction. We further expect females to preferentially establish strong affiliative relationships with related partners.

MATERIALS AND METHODS

Study Population

Data were collected from a free-living population of house mice living in a building located in the vicinity of Zurich, Switzerland. The population inhabited an old barn ($72m^2$) divided into four equal sections by large plastic walls, and each section contained 10 nest boxes and various shelters distributed across the section (totalling 40 nest boxes over the entire barn). There were holes in the dividing walls enabling the mice access to all areas, and wooden and plastic structures throughout the barn allowed the mice to form and defend territories. The system was closed to larger predators but open to dispersal and immigration of the mice, as well

as parasites and diseases (Dobay et al. 2015; Origgi et al. 2015). When the mice leave the barn they are subject to predation by larger predators, such as cats, foxes and birds of prey. Food (50/50 mixture of oats and hamster food, Landi AG, Switzerland) and water were provided ad libitum in 3 feeding trays and 4 water dispensers per section.

During the study period 2008 to 2009 all individuals were captured every 7 weeks, with an average of (mean ± SE) 104.5 ± 10 adult mice, 63.6 ± 9 sub-adults and 37.6 ± 10 pups, mice recorded in such an event. Individuals were considered adult (and potentially reproductively active) at a body weight of 18g, when they were implanted with a subcutaneous transponder between the shoulder blades using a sterile needle (RFID tag; Trovan-ID-100A implantable micro-transponder: 0.1 g weight, 11.5mm length, 2.1mm diameter; implanter Trovan IID100E; Euro ID Identifikationssysteme GmbH & Co, Germany). During capture a small tissue sample was taken from the ear for genetic analysis (ear puncher Napox KN-293: 1.5 mm diameter). Females in the study population very rarely get pregnant or lactate before they reach a body weight of 18g, although males and females may occasionally reproduce at a lower body weight which is revealed by genetic analysis based on samples taken from dead and weaned pups.

There have been no adverse effects reported from the transponders used in this population or the literature. The Swiss Federal Law on Animal Protection recommends ear tissue samples for use in genetic analysis. More detailed information about the tagging procedure; set-up and population can be found in Auclair et al. (2014a) and König & Lindholm (2012) respectively. Data collection was approved by the Veterinary Office Zurich, Switzerland, no. 215/2006.

Monitoring of Interactions

Once tagged, individuals had a unique identification number that enabled for non-invasive monitoring of their position in the barn, either using a hand-held transponder reader (during handling or when mice were inside nest boxes or shelters) or by an automatic antenna system. The entrance tube of every nest box in the barn was fitted with two antennas (NewBehavior AG, Zurich, Switzerland), which allowed for the continuous monitoring of all individuals that visited each nest box, and to determine all encounters between individuals. For a detailed description of the antenna system and remote monitoring see König et al. (2015). We assessed a focal female's nest box use (the location and number of nest boxes a mouse visited) for every month during the six-month period after she was tagged. Additionally, on a monthly basis we determined for each focal female her dyadic encounters. The antenna system enabled us to calculate the total number of meetings and the duration of these meetings between a focal female and all of the mice (here meaning all tagged adult males and females) she met. In this study we focused on all females that were tagged from January 2008 through to December 2009, and were recorded by the antenna system for a complete six months after receiving a transponder (data from the antenna system extends to the beginning of July 2010).

Composite Sociality Index

To investigate the strength of social interactions between female dyads and determine if females associated with particular females more often than others, suggesting a social preference, we used a technique described by Silk et al. (2006a; 2006b; 2013) to compute a composite sociality index (CSI). The CSI is based on the relative frequencies of positive interactions between individuals, and was used to measure the strength of a dyad's affiliative relationship (Silk et al. 2013). With this index we were able to calculate the extent to which

each dyad deviated in its association time from the average female dyad in their social group during a given month (Silk et al. 2006a; 2006b; Kalbitz et al. 2016). Unlike other studies that have used such an index, we did not use grooming as a measure of affiliation, due to lack of visual access to nest boxes. Instead, we used the frequency of meetings between female dyads (a focal female and all females she interacted with per month) and the duration of time spent together in the same nest box.

The CSI was calculated as follows, the values for association time and number of meetings for each focal female dyad (*ij*) were divided by the mean value of the specified behaviour across all female dyads in the same social group:

$$CSI = \frac{\frac{A_{ij}}{A_{ave}} + \frac{M_{ij}}{M_{ave}}}{2}$$

In this equation, A represents the total time a female dyad (*ij*) spent together in a nest box during a given month (association time); A_{ave} represents the average association time of all female dyads within the same social group for the same month; M represents the total number of meetings in a nest box that occurred during a given month between a dyad; and M_{ave} represents the average number of meetings between all female dyads of the same social group. The average CSI across all dyads was by definition 1. When values were > 1 they indicated that a dyad had a strong affiliative relationship, stronger than the average associations in the group, but values between zero and one (excluding 1) suggested the relationship was not characterised by pronounced amicable interactions, and thus did not reflect a preference for that partner (Silk et al. 2006a; 2006b; Kalbitz et al. 2016).

Partner Stability

We were further interested to determine whether females had consistency in their top partner(s) from one month to the next, to determine if females consistently interacted with the same partner(s) over longer time periods. Size and composition of groups may change in house mice populations due to mortality, immigration or emigration. We therefore determined the consistency of preferences and controlled for the possibility of a partner disappearing by using an equation adapted by Cheney et al. (2012).

First, we ranked the CSI scores for each month from highest to lowest per focal female; we then determined the average CSI scores for each rank across all dyads. This allowed us to decide how many individuals we should consider as a top partner (in primate studies three top partners are commonly used; Cheney et al. 2012; Silk et al. 2012; Kalbitz et al. 2016), in our case we used the ranks that had average CSI values above one, which were the partners ranked 1 to 4 (top four partners), and used these top four partners for further analysis. For all focal females that were monitored for the entire six months in the barn, we were then able to assess the consistency of their top partners using a partner stability index (PSI) with the equation:

$$PSI = \frac{NS - U}{NS - S - X}$$

Where N is the number of months in which partner choice was evaluated, S is the number of top partners being evaluated, U is the number of different females that occurred in a focal female's top partners during the 6 months monitored, and X is the number of top partners that

disappeared from one month to the next. Here, $N = 6$ and $S = 4$ so the equation can be simplified to $(24 - U / 20 - X)$.

The important aspect was that this index controlled for changes in a female's top partners (X) that were related to potential dispersal or deaths, a new-tagged female could also have joined the group. Values of PSI equal to 1 suggested a female had the same top partners for the entire time, and values of 0 suggested a female's top partners changed every month. Alternatively, low values also imply that a female switched partners every month even when all previous preferred partners were still present, suggesting a lack of social preference. Values greater than one were also possible if a female had many different top partners and the majority of these disappeared, this could imply that a female's social group was unstable. An example is provided in Table 1, where female 'C561' had 7 different top female partners, of which 2 disappeared during the 6 months (they were never recorded again by the hand-held readers or by the antenna recording device during the six-month monitoring period in the home area of interest); therefore, her PSI score was 0.944 (equation: 24 – 7 / 20 – 2). We also used this equation to calculate the stability of focal female's partners that had lower ranks; each rank class consisted of four ranks (rank classes: 5 – 8, 9 – 12 and 13 +). We could then determine if stability of a focal female's partners differed by rank class.

Table 1. Example of how the partner stability index (PSI) was computed for the female mice. Female identity (ID) is given as the last 4 characters of their tag number.

Female C561	Top partners			
	1	2	3	4
Month 1	CC8C	57BE*	574D	D1A1
Month 2	CC8C	E2FD	574D*	D1A1
Month 3	4DAD	E2FD	CC8C	D45A
Month 4	4DAD	E2FD	CC8C	D45A
Month 5	E2FD	4DAD	CC8C	D45A
Month 6	4DAD	CC8C	D45A	E2FD

* Females that disappeared and were no longer present as an interaction partner in the home area of interest, they either dispersed or died. An asterisk next to the ID indicates the last month this individual appeared in the social group. The different partner IDs are shaded in grey, the first time they appear in a focal female's top partners (see text for explanation).

In 3 cases the focal female's PSI was greater than 1 because she had many different top partners and many partners that disappeared, or this could suggest that the focal female moved groups regularly. Under such a scenario we would predict that the focal female's social partners were not stable. However, in these instances we could not be certain, and therefore, these 3 focals were excluded.

Reproduction

Reproduction was closely monitored in the barn whereby all litters found were recorded. Every 8 to 12 days a nest check was carried out where nest boxes and shelters were searched for new litters born (mice very rarely give birth to litters outside of nest boxes). Nest boxes could be opened at the top allowing experimenters access inside to examine the litters. Before opening a nest box we used a hand-held transponder reader to note down all tagged adult mice present inside. On finding a litter we determined litter size and the age of pups using morphological indicators (such as skin coloration, fur growth and teeth eruption, allowing age to be estimated at ± 1 day, day 1 was considered day of birth: Auclair et al. 2014a; König and

Lindholm 2012). When pups were estimated to be 13 days old they were searched for again and an ear tissue sample and morphological measurements were taken for each pup. This age was chosen as it is the closest age to weaning in which a tissue sample can be reliably collected; as pups open their eyes at day 14, meaning they can more easily escape (weaning commences at day 17 and ceases at day 21 – 23; König and Markl 1987).

Ear tissue samples were also taken from all handled adults and corpses allowing a mother and father to be assigned to sampled individuals. Using the technique described in Auclair et al. (2014a), DNA was amplified at 25 microsatellite loci using a 95% confidence level in Cervus 3.0 (Marshall et al. 1998), success at assigning a mother to pups was 87 – 88%. For the purpose of our study we recorded the number of litters (at least one pup sampled when 13 days old) for each focal female during the six-months of monitoring. This enabled us to determine whether factors of a female's social environment influenced the number of litters they weaned during the time period being monitored.

For pairwise relatedness estimation we compared how genetically similar two individuals were to each other to the average similarity between dyads of the year the focal female was tagged (either 2008 or 2009) at 25 microsatellite loci. To find out the most suitable estimator to use, we took from our pedigree, 50 full sibling and 50 parent-offspring dyads (expected relatedness r = 0.5), 50 half-sibling dyads (expected r = 0.25), and 50 dyads of unrelated individuals (expected r = 0; living simultaneously and not sharing a grandparent). We estimated pairwise relatedness values for all dyads using seven different estimates for r and correlated them against the pedigree r values using Coancestry (Wang 2011). The results determined that the Wang estimate (Wang 2002) had the highest correlation (R = 0.80) between pedigree r and estimated r, and therefore we used this estimate to calculate pairwise relatedness in the current study.

Statistical Analyses

Statistical analysis were carried out using R version 3.1.3 (R Core Team 2015) with add on package 'lme4' (Bates et al. 2014). We used a linear mixed model (LMM) to assess if CSI was influenced by pairwise relatedness and age difference between the females of a dyad. Group identity, dyad identity and number of months the dyad were co-resident in the same social group (within the six month observation period) were included as random effects. After visually inspecting the model assumptions we determined the residuals were not normally distributed (Zuur et al. 2009). Therefore, we used box-cox methods to determine which transformation was required (with the 'car' package in R; Crawley 2007; Fox and Weisberg 2011), and found a square root transformation of CSI was necessary.

We additionally used a LMM to assess factors influencing the PSI of a female's top four partners over the six months. Here the average number of different females in the focal female's social group, the average number of nest boxes the focal female used, whether the focal female was reproducing during the 6-month period, average relatedness of her top four partners (average over monthly means), average age difference of her top four partners (average over monthly means), the total number of different females the focal female met (during the six-months) and the total number of different males the focal female met (during the six-months), were included as explanatory factors. The age the focal female was tagged was included as a random effect. To assess differences in PSI scores by the different rank classes (1 – 4, 5 – 8, 9 – 12, and 13 +) we used a LMM, whereby rank class was included as an explanatory variable and identity of the focal female was included as a random effect. PSI values were bounded by 0 and 1, therefore we used a logit transformation (Warton and Hui 2011). To find out whether there

were any differences in PSI scores by rank class, we used a *post hoc* test with manually assigned contrasts (using the 'multcomp' package in R; Hothorn et al. 2008).

A generalized linear mixed model (GLMM) with Poisson error distribution was used to investigate factors influencing the number of months a female dyad was co-resident in the same social group; pairwise relatedness and age difference between females were included as explanatory variables. To control for any dependencies in the data we included group identity and number of females in the social group (average for the six months) as random effects. Due to age difference between the dyads being on a different scale to the pairwise relatedness values, we were required to centre and scale the age difference data for simpler comparisons and a better model fit - this did not change the spread of the data (Schielzeth 2010).

We further used a generalized linear model (GLM) to test the prediction that reproduction would be higher when a female's partner stability was high; we included the number of litters weaned in six months as the response variable. Alongside PSI we included the age the focal female was tagged, the number of different top partners a focal female had, the average number of females in her social group (average over the six month period), the season (winter: September to February; Summer: March to August; seasons were defined to match the difference in reproductive periods, reproduction is highest during summer and lowest during winter) she was tagged, and the total number of different male partners met, as explanatory variables. Given that the data were over dispersed, we used a quasipoisson error distribution.

For all linear models significance of explanatory terms was determined using likelihood ratio tests (LRT; Crawley 2007), via stepwise removal of terms, thus models with all explanatory variables were compared to models with the explanatory variable of interest removed, until the most adequate model was found. In the results we include P values for significant factors from the most adequate model only; for all other terms $P > 0.05$, and therefore they are not stated.

RESULTS

During the two-year study period 211 female mice were tagged, 143 females reproduced during their time in the barn (gave birth to pups that survived until sampling at 13 days old), and 68 females did not reproduce before they died or dispersed. For the purpose of the study we focused on females that were read by the antenna system for a complete six months following the injection of a transponder. Eighty-seven tagged females fell into this criterion, resulting in 1, 947 unique dyadic relationships between females in the barn. During this six-month time period we also recorded the occurrence of reproduction and determined that 65 females had at least one litter and 22 females had no litters (these females could have had litters outside of the six-month monitoring period). Table 2 summarises the different attributes of the focal females, their nest box use and number of interaction partners.

Table 2. A summary of factors observed during the six months for the 87 focal females that were monitored. Numbers are given as total over a focal female's observation time, with the exception of the number of females/individuals in a social group, and number of nest boxes used, which is given as a monthly mean.

	Mean ± SE	Range
Number of nest boxes used	7.41 ± 0.26	3, 15
Number of different females met	23.08 ± 0.77	6, 38
Number of different males met	23.74 ± 1.01	7, 61
Number of individuals in a social group	20.31 ± 0.75	7, 38
Number of females in a social group	11.79 ± 0.45	3, 21
Number of different top partners	9.38 ± 0.23	5, 15
Number of litters weaned in six months	2.04 ± 0.18	0, 5
Age at tagging (d)	142.10 ± 9.01	54, 387
Age of focal female had her first litter (d)	190.53 ± 11.27	40, 425

Variation in Sociality

We calculated the CSI for all 87 focal females and their female interaction partners across the six months monitored. The distribution of this index shows how social contact varied across all female dyads in the barn during this time period. The CSI distribution is skewed to the right, and the mean is defined as 1 (Silk et al. 2006a; 2006b). The median is 0.89 and the index exceeded one for 44.7% of dyads, and two for 10.3% of dyads (Fig. 1). This implied that more than half of the dyads had relatively weak associations (55.3%), and a small proportion of dyads had noticeably strong affiliative associations.

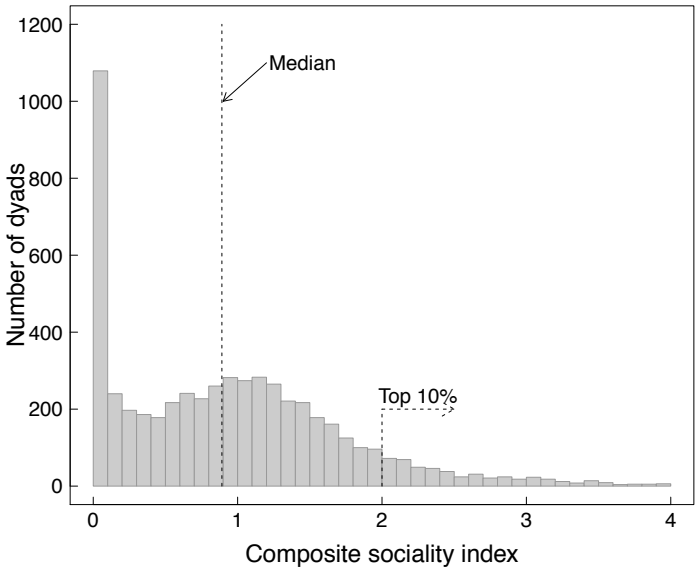

Figure 1. Frequency of different composite sociality (CSI) scores for all dyads monitored. Here the figure illustrates the distribution of CSI values ($0 < CSI \leq 4$), 80 dyads (1.4%) had scores greater than 4, these are not shown here. The median is shown, and a second dashed line (further to the right of the median) indicates that only 10% of the dyads had values above 2.

Females had stronger affiliative associations with females that were more closely related to them, whereby CSI scores were significantly higher when a female pair was more related (χ_1^2 = 310.3, P < 0.001, Fig. 2). We found no significant effect of age difference between the females on CSI scores, suggesting that the age cohort of a female's interaction partners was varied.

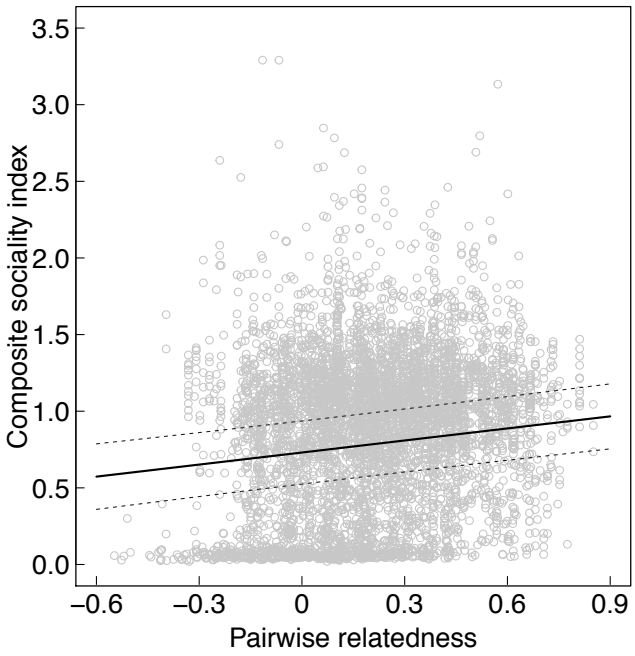

Figure 2. A plot illustrating that dyads with higher pairwise relatedness associated more often, they had higher CSI scores (here shown as square root CSI). Line ± 95% CI was determined using the model predictions

Duration of Co-residence

We looked at the number of months that dyads were co-resident, defined as the time period when a focal female and another female had social interactions in shared nest boxes for the six months the focal female was monitored (Fig. 3). A high proportion of female dyads were only co-resident for one month, however, 22% were co-resident for the full six months. Not all female partners were present during all six months of a focal female's monitoring, 47.7% disappeared (died or dispersed).

Pairwise relatedness had a significant effect on the duration that females' were co-resident (χ_1^2 = 348.5, P < 0.001), whereby females resided together for longer when their pairwise relatedness was higher. As CSI and relatedness were correlated, and female dyads with higher relatedness had higher CSI scores (Fig. 2), then CSI should also be higher in dyads that co-resided for longer. Females that were co-resident for less than six months include both females that disappeared and females that joined a focal females group.

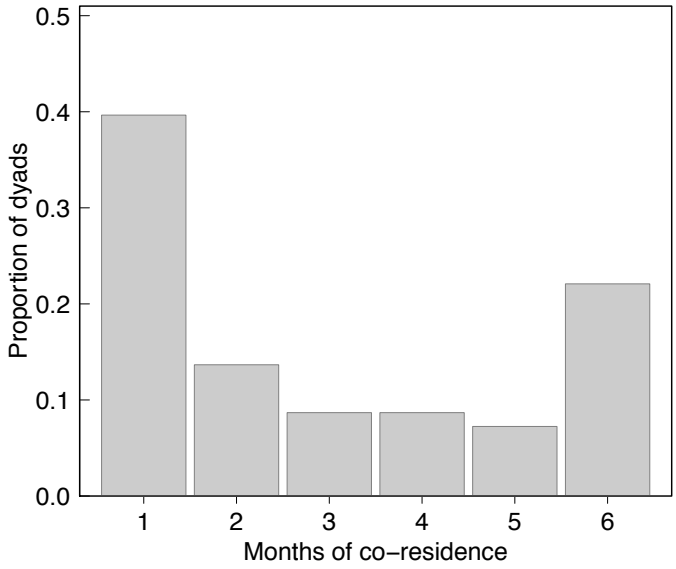

Figure 3. Co-resident female dyads for the 6 months a focal female was monitored, here shown is the proportion of all female dyads by length of co-residence.

Stability of Partners

We calculated PSI values to estimate the stability of a focal female's four top partners over the six-month observation period. Here values for PSI ranged from 0 to 1, and number of different top partners ranged from 5 to 15 (Table 2). The average PSI for the top four partners across all focal females was 0.850 ± 0.01 (mean ± SE). There was a significant effect of rank class on PSI values (χ_3^2 = 77.71, $P < 0.001$, Fig. 4), whereby stability of preferred partners decreased by rank class. *Post hoc* tests revealed that the PSI scores of partners ranked 1 - 4 were significantly higher than those of partners ranked 5 - 8 (z = 5.40, $P < 0.001$), there was no significant difference in PSI scores between partners ranked 5 – 8 and 9 - 12 (z = -0.21, P = 0.994), but partners ranked above 12 had significantly lower PSI scores that those ranked 9 - 12 (z = 1.08, $P < 0.001$). Among all females sharing a social group, 41.5% appeared as a focal female's top four partner during at least one month.

We further assessed whether the PSI of a female's top four partners was affected by group size, more specifically, by the average number of females present each month in her social group. Females had significantly higher PSI scores when the number of female interacting partners in her social group was lower (χ_1^2 = 40.13, P = 0.001). PSI was not significantly influenced by average pairwise relatedness or the average age difference between the focal and the top four partners (an average over the six-months), or any other factors tested in the model.

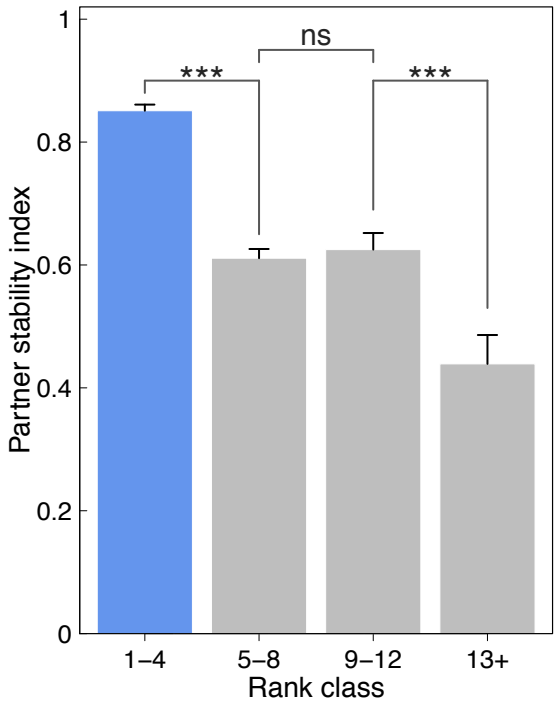

Figure 4. Partner stability index (PSI) for the different categories of female partners (rank classes), the highest rank is given to the partner of a focal female with the highest CSI score. The blue bar represents the PSI of a focal female's top four partners, and the grey bars illustrate the stability of female dyads for the lower rank classes. Bars represent mean ± SE, and *** ≤ 0.001.

Reproduction

We expected that the stability of a focal female's interaction partners, reflected by high PSI scores, would improve a female's probability to produce a litter during the time period being monitored. However, there was no significant effect of PSI, relatedness or age difference between the females on reproduction. Instead, the number of successful litters was significantly higher if a focal female's social group was smaller (χ_1^2 = 17.63, P < 0.001), meaning a female gave birth to more litters when the number of females in her group was lower. Unsurprisingly, females also had significantly fewer litters if they were tagged (beginning of monitoring period) in winter than those tagged in summer (χ_1^2 = 5.61, P = 0.022).

DISCUSSION

In this study we aimed to determine whether free-living female house mice socialised with specific female partners within their home area, and whether these partners were stable over a six-month monitoring period. In order to monitor female movements they were injected with a transponder at a body weight of no less that 18 g and were on average 140 days old. Using this movement data, we found that females interacted each month with on average 12 females, and a focal female's home area consisted of on average seven nest boxes. Composition of social groups varied over time, due to mice dying or disappearing from the group, or the joining of a new female (most likely after a population monitoring event, when new females were tagged). As a consequence females interacted with up to 38 different tagged adult females and 61 different tagged adult males during the six months they were monitored. Within such groups adult females modified their pattern of interactions and expressed social preferences

among group members. Therefore, female house mice in our study proved to live in a rather complex social environment. Upon examining their social interactions we found that the strength and duration of female dyadic associations were influenced by pairwise relatedness, whereby females associated more often, and co-resided for longer, with female partners who were more closely related to them. We further found that female dyads were relatively stable over time, and that having more females in a social group reduced the stability of these associations. Females had more litters within a six-month monitoring period if they had reached reproductive age (body weight ≥ 18 g) in summer, and when a focal female's social group consisted of fewer female interaction partners. Taken together, these findings suggest that female house mice form social preferences towards specific partners in their social environment, and that these social partners are generally related and consistent over time.

Do strong affiliative relationships reflect partner preferences?

We quantified the strength and duration of 87 focal females' dyadic associations, over a six-month period in a free-living population, through use of CSI scores. Interestingly, females sharing the same home area (overlapping nest boxes) did not all interact at a similar rate; instead females spent more time with some group members than others. Similarly, Weidt et al. (2008) demonstrated in laboratory enclosures that the frequency of meetings differs between dyads, and a preference is defined when females are found together more often than expected by chance. Associations between individuals is often used as an indicator for a social preference, for example female giraffes, *Giraffa camelopardalis*, showed significant preferred relationships with other females, which were explained to some degree by spatial overlap and relatedness (Carter et al. 2012). Furthermore, female chimpanzee dyadic association rates indicated affiliation among spatially close partners (Foerster et al. 2015), and female chacma baboons form strong and consistent social associations with other females in their social group (Silk et al. 2010a). In the current study, approximately 45% of our female dyads had associations that were stronger than the average of all dyads, and 10% had noticeably strong affiliations with females that shared the same nesting sites. Our findings, alongside those of other studies, indicate that females sharing the same social environment can form affiliative relationships or preferences for certain conspecifics. However, we did observe that females disappeared or died during the six-month study period, the reason for these disappearances are not clear. Future studies are required to disentangle the processes behind dispersal and emigration, and may reveal whether they are induced by specific social interactions between same-sex or opposite-sex partners.

Partner Stability and Reproduction

PSI was relatively high for the females' top partners, suggesting females socialised with the same partners across different months. Social preferences among female partners have been demonstrated to result in fitness benefits, for example higher social integration among female feral horses results in increased foal birth and survival rates (Cameron et al. 2009), and in chacma baboons increased life span (Silk et al. 2010a), and increased offspring survival (Silk et al. 2003) have been linked to the stability of social bonds among females. Furthermore, female house mice under laboratory conditions exhibited preferences for unfamiliar partners that led to increased lifetime reproductive success (Weidt et al. 2008). In the current study our purpose was to verify the existence of female preferences in a free-living population of house mice. We found no effect of PSI on number of weaned litters; however, here we focused on whether at

least one pup from a litter survived to sampling age (13 days old). We used this measure of litter success as a proxy to assess whether strong associations contribute towards successful reproduction, which may signal that cooperation is influenced by the female social interactions. In our study population, litter size at birth is difficult to accurately quantify, as to minimise invasive disturbance, nest boxes are not checked daily for new litters (litters are typically found when pups are a few days old). Thus, we provide preliminary evidence that individualised preferences occur among female house mice; but in order to make parallel comparisons to the study by Weidt et al. (2008), and those of Silk et al. (2003, 2010b), future studies could examine the long-term implications of social relationships on lifetime reproductive success and longevity.

PSI was however influenced by the number of females in a social group, whereby females with fewer different female interaction partners had higher PSI scores than those with more female partners. Having fewer female interaction partners could have allowed female's to maintain their social preferences more easily, due to reduced competition over resources or breeding opportunities. The suggestion that females had more breeding opportunities is supported by the finding that females with fewer female interaction partners in their social group weaned more litters in six months. Additionally, the number of males a female interacted with had no effect on the number of litters weaned, suggesting that females were not limited in access to males. Armitage and Schwartz (2000) showed that female marmots, *Marmota flaviventris*, that live in exceptionally large groups have less successful reproduction than females living in intermediate sized groups, and in tuco-tucos, *Ctenomys sociabilis,* group size had a direct effect on reproductive performance, whereby increases in reproductive success were associated with decreasing group size (Lacey 2004). When there are more females around reproductive success may be influenced by resource competition, as more females results in lower food availability which in turn can lead to an impaired nutritional state, or the increased harassment from other females may lead to elevated stress levels (Armitage and Schwartz 2000; reviewed in Silk 2007). Therefore, differences experienced by females in terms of reproductive performance may be dependent on the size and structure of their social group.

Female Preferences and Relatedness

We found that females associated more often with females that were more closely related to them, suggesting that their home area was composed of related females. Previous findings from other studies support this suggestion as females were shown to share nest boxes with related individuals (König et al. 2015; Harrison et al. in prep). These findings compliment the suggestion that females are the philopatric sex (Gerlach 1990; Dobson et al. 2000; Rusu and Krackow 2004), suggesting dispersal is limited and there is an increased the chance that social interactions will occur among related individuals (Hamilton 1964a; 1964b; 1970). House mice have further been shown to typically nest with kin (Wilkinson and Baker 1988), and were more successful reproductively when they associated spatially with related females (Dobson et al. 2000; Rusu and Krackow 2004), while communally nursing. Female house mice have also been shown to prefer partners that shared allelic forms of the major histocompatibility complex (MHC) gene (Manning et al. 1992), or those that shared own major urinary protein (MUP) genotype (Green et al. 2015). However, we can not be certain of how females differentiate between interaction partners, they may use familiarity to recognise kin via phenotype matching (Hauber and Sherman 2001), or assess relatedness at specific loci such as MHC or MUP similarities (Manning et al. 1992; Roberts and Gosling 2003; Green et al. 2015) instead of at the

genome wide level, such information is difficult to disentangle in a free-living environment as there are often many confounding factors.

However, socialising or living with related conspecifics can lead to many advantages, such as assistance in finding or defending of mates and territories, cooperation when rearing offspring or when foraging, and decreased predation risk (Clutton-Brock 2006; Silk 2007; Shultz and Dunbar 2010; Seyfarth and Cheney 2012). Studies in voles, *Microtus townsendii*, have shown that females living close to kin reared higher numbers of offspring than those that resided with non-kin (Kawata 1990), and individuals that settled close to kin had a higher probability of surviving from one year to the next (Lambin and Krebs 1993). It has further been suggested that associations among kin can enhance a female mouse's reproductive performance (Sutherland et al. 2005), and even lead to egalitarian reproduction (Dobson et al. 2000). Additionally, in laboratory experiments, house mice that communally nurse with related females have higher lifetime reproductive success than when communally nursing with unrelated females (König 1994a; 1994b). As females in our study associated more regularly with a related partner, when it came to reproduction they would have a higher chance of nursing communally with a related individual, which in turn would lead to increased fitness benefits. Taken together, this could suggest that in socialising with related partners, females in this study were increasing their chance of successfully reproducing and potentially decreasing infanticide vulnerability.

Conclusions

Our results on free-living female house mice coincide with observations from a laboratory study on wild-derived females kept in enclosures (Weidt et al. 2008), whereby sexually mature females were social, and established strong affiliative relationships with specific female partners. Relatedness and group size prominently affected the strength of social interactions and the stability of partner preferences respectively, which is interesting given that females cooperate with other females in their home area during communal nursing of litters (Weidt et al. 2014; Harrison et al. in prep). Females associated more often, and co-resided for longer, with another female if she was closely related. Furthermore, the stability of such dyadic associations was stronger in smaller groups with few female social partners, and within such groups females reproduced more often during a six-month time period. Future studies would be required to better understand the consequences of these preferences on female social structure, lifetime reproductive success and longevity. In sum, our results coincide with predictions that reveal cooperation is facilitated in small groups, and when interacting with relatives (reviewed in Silk 2007).

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CHAPTER 3

Oxytocin and Social Preference in Female House Mice *(Mus musculus domesticus)*

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Oxytocin and Social Preference in Female House Mice (*Mus musculus domesticus*)

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ABSTRACT

In social species, same sex-individuals may form social bonds behaviourally expressed as individual preferences, resulting in fitness benefits such as increased offspring survival, longevity and group cohesion. As a result of individual preferences, female house mice (*Mus musculus domesticus)* form social affiliations while communally nursing, and may do so with kin or non-kin. However, the mechanisms behind the formation of such preferences are unknown. Oxytocin has been linked to a range of social behaviours including bond facilitation, social memory and parental care. Here we experimentally increased oxytocin in pairs of unfamiliar, unrelated females and predicted that females with elevated oxytocin would demonstrate increased affiliative behaviours compared against a control. Subsequently we tested for the formation of a social preference, using a preference test with the previous partner and a new unfamiliar female. Our results indicated no significant effect of treatment on positive and negative behaviours between females during the three initial cohabitation days. In both treatments females demonstrated increased socio-positive behaviours and cohabitation time with their partner, and decreased socio-negative behaviours and latency to meet, over the three-day period. During the partner preference test, control but not oxytocin females demonstrated a significant preference for their cohabitation partner, oxytocin females spent similar amounts of time with both stimulus females. Therefore, increasing peripheral oxytocin appears not to be involved in the facilitation of initial encounters with a stranger but may hinder the formation of a preference for this new partner.

INTRODUCTION

Same-sex individuals that establish bonds with their conspecifics can benefit from increased offspring survival, longevity, decreased stress and increased group cohesion (König 1994; Silk et al. 2003; Yee et al. 2008; Seyfarth & Cheney 2012). Typically, time spent in close association has been considered as an indicator of social bonding between individuals, and benefits have been documented in a wide variety of species including female savannah baboons (*Papio cynocephalus*) who gain greater offspring survivorship when individuals are more socially integrated (Silk et al. 2003), and female house mice (*Mus musculus domesticus*) who when nursing with a preferred female partner have greater reproductive success (Weidt et al. 2008). Positive correlates between sociality and reproductive success have also been found in female horses, *Equus equus* (Cameron et al. 2009), bottlenose dolphins, *Tursiops truncatus* (Frère et al. 2010) and male macaques, *Macaca assamensis* (Schülke et al. 2010). These findings, among others, have demonstrated and brought to light the benefits associated with sociality and bonding with same-sex conspecifics. Therefore, in order to benefit from such fitness advantages, social partnerships are believed to be important.

The mechanisms behind choosing a social partner and establishing social partnerships are poorly understood, however, a number of processes may be involved. One such mechanism is the use of physical and physiological cues in which a conspecific's relatedness, breeding status or ability to produce offspring (Hurst 1990; Weidt et al. 2008) could be determined. Relationships between non-kin have been linked to endocrinological mechanisms, in particular that of the oxytocinergic system (Beery & Zucker 2010; Wittig et al. 2014). In humans and other mammals, the peptide hormone oxytocin has been linked to a range of social behaviours including the facilitation of bonds between a mother and her offspring and between mating partners (reviewed in: Anacker & Beery 2013). In particular, oxytocin has been extensively studied for its effects on pair bonding in the monogamous prairie vole, where it was demonstrated to play a role in facilitating pre-copulatory bonding between males and females and increased social contact (Williams et al. 1994; Insel and Hulihan 1995; Cho et al. 1999; Ross & Young 2009).

Increased interest in oxytocin over recent years has led to a variety of studies related to social behaviours. Manipulation of oxytocin levels by injection increased huddling behaviour of females towards an unfamiliar female in meadow voles, *Microtus pennsylvanicus* (Beery & Zucker 2010); increased a range of cooperative behaviours such as digging, guarding and pup feeding in meerkats, *Suricata suricatta* (Madden & Clutton-Brock 2011); and increased investigatory behaviour and time in close proximity with familiar conspecifics, in naked mole rats, *Heterocephalus glaber* (Mooney et al. 2014). Additionally, elevated levels of oxytocin were found in chimpanzee, *Pan troglodytes*, urine following grooming behaviour with preferred social partners (Crockford et al. 2013), and after food sharing with conspecifics (Wittig et al. 2014). These studies, among others suggest that oxytocin aids in facilitating bonds between known individuals and acts to strengthen these relationships. Conversely, various studies in oxytocin knockout mice caused a lack of social memory (Ferguson et al. 2000, 2001) and increased aggression (Winslow & Insel 2002). Therefore, oxytocin is a prime candidate to study its role in choice of same sex social partners.

House mice offer an ideal study species to investigate choice of social partner as they are included in a small percentage of mammals that rear their offspring via communal nursing, when two or more females cooperate and indisciminately nurse their offspring in the same nest (König 1989; Packer et al. 1992; Hayes 2000). Research has shown that female mice preferentially nest with familiar sisters forming egalitarian relationships that increase lifetime

reproductive success for both females (König 1994). Females also form preferences for individuals when kept in groups of unrelated females, where they will establish communal nests and have greater success when nursing with a preferred partner (Weidt et al. 2008). Therefore, it is believed that females choose social partners to communally nurse with and do so when the most suitable partner is available. Females in a free-living environment are also selective in their partner choice and when given the option only choose to nest communally in 33% of cases (Weidt et al. 2014). In semi-natural, outdoor enclosures communal nursing occurs in up to 90% of cases (Manning et al. 1995). Time spent together before communally nursing is suggested to be the best indicator for partner preference (Weidt et al. 2008). However, regardless of whether or not females nest with kin or non-kin the mechanisms involved in choosing a communal nursing partner are not yet known.

In this study we experimentally increased oxytocin, over a period of three days, in pairs of unrelated, unfamiliar females and predicted that females with elevated oxytocin would demonstrate increased affiliative behaviours when compared against a control. Subsequently, we tested for the formation of a social preference using a preference test, with a choice between the previous partner and another unfamiliar female. We thus aimed to understand whether oxytocin could influence the initial behaviours females exhibit towards an unfamiliar female, and determine if it can facilitate establishment of a preference.

METHODS

Subjects

We used 48 female house mice that were sexually mature but non-breeding (virgin). Animals were laboratory born F1 to F3 descendants of wild house mice (*M. musculus domesticus*) from a barn population near Zurich, as described in König and Lindholm (2012). Animals were weaned at day 23 and kept in same-sex sibling groups where they remained until 8 - 10 weeks of age; in rodents the oxytocin system is fully developed at weaning (Yamamoto et al. 2004). Each cage contained standard animal bedding (Lignocel Hygienic Animal bedding, JRS), with *ad libitum* cardboard and tissue for bedding and shelter. Mice were kept at a temperature of 22-24°C and humidity of 50-55%, under a constant light dark cycle of 14:10 hours (lights on at 05.30 h CET, with a half hour dawn and dusk phase at the beginning and end of the light phase). At all stages food (laboratory animal diet for mice, Provimi Kliba SA, Kaiseraugst, Switzerland) and water was provided *ad libitum*. To avoid excessive manipulations, we did not check for oestrous cycles or ovariectomize the females. When wild derived female mice are housed in the conditions described above, 70% of females do not show ovarian cycles (Weidt 2007). Given that females were randomly assigned to the different injection treatments, endogenous oestrogen levels should be low and overall similar between treatments. Animal use and experimental design were approved by the Veterinary Office Zürich, Switzerland (Kantonales Veterinäramt, Zürich, no. 34/2014).

Experimental Procedures

In total, the experiment lasted for five days, which comprised of a cohabitation phase lasting three days followed by a 7-8 hour separation period and a one-day preference test.

Phase A: Cohabitation

Pairs of unfamiliar genetically unrelated females were randomly assigned to one of two groups, oxytocin (OT) or saline control (CON); both females in a pair received the same treatment. Unfamiliar, unrelated females were chosen, as we wanted to understand what mechanisms promote initial preference formation; familiar or related females could have established a preference prior to the start of the experiment. Female pairs were matched, where possible, in age (age difference: 7 ± 10 d, mean ± SE) and weight (weight difference: 2.9 ± 1.3 g, mean ± SE). Injections were given on 3 consecutive days between 16.00 h and 18.00 h prior to the dark phase (lights out at 20.30 h, during dusk and dawn mice are most active, Mackintosh 1981). In order to keep stress to a minimum all females were restrained in a secure, one-hand technique. Due to all females experiencing the same restraint we believe any stress effects on behaviour should be similar for all females. Following each injection females were allowed a fifteen-minute recovery period in a Makrolon Type II cage (width: 18 cm, length: 24 cm, height: 14 cm; made of transparent polycarbonate plastic), afterward the cages of the pair being observed were connected with a transparent plastic tube, allowing both females access to both cages. As soon as both cages were connected behaviours were video recorded (Sony camcorder) for later analysis, with red light allowing video recordings during the dark phase (20.30 h to 06.30 h).

Using the video footage a series of observations were carried out. Latency for the two females to meet was recorded once the cages were joined, and after the first interaction a 60-minute behavioural focal followed. In addition, twelve 10-minute behavioural focals were made at the beginning of every hour (19.00 h to 06.00 h). The longer initial focal observation was chosen based on the results obtained by Neumann et al. (2013), where a peripheral OT injection led to elevated circulating OT for the first two hours post administration. All occurrences of behaviours and their duration were recorded for the pair together. For analysis these behaviours were grouped into four main categories: socio-positive, socio-negative, neutral behaviours and time in the same cage (see Table 1, for detailed behavioural descriptions). As negative interactions occurred quickly (lasting less than a minute) we chose to analyse counts of negative interactions and time of positive interactions (as duration of positive behaviours lasted for longer periods and provided a more accurate measure). Time spent in the same cage was also analysed and scoring was completed blind to treatment group. Additionally, we recorded whether the females were in the same or different cage on every half hour throughout the night, and at three time points (09.00 h, 12.00 h and 16.00 h) during day light hours.

Table 1. Description of socio-positive, –negative and neutral behaviours recorded during cohabitation, behavioural observations

Behaviour	Description
Socio-positive	
Huddle/Rest	Resting in side by side contact
Allogroom	Grooming, being groomed, or both females groom each other
Side by side contact	Eating or climbing side by side, not resting or huddling
Investigatory	Sniffing other; Nose, Ano-genital or elsewhere (positive when not followed by chase or fight)
Follow	Following the other (walking), female being followed is not running
Socio-negative	
Chase	Pursuing the other (running) or aggressively running towards other
Flee	Rapidly moving away from other (running)
Fight	Bite or attacking other
Submissive	One female to the other, rearing on hind legs
Tail rattling	Rapid side to side movement of the tail
Neutral	
Rest separately	Both resting in the same cage as each other, no body contact
No interactions	Both in same cage but no interaction e.g. one in cardboard shelter, other active

Peptide and Doses

Synthetic oxytocin (Product: O4375 - 250 IU, Sigma Aldrich Co., Germany) was dissolved in sterile saline (0.9% NaCl, Bischel) to give a concentration of 0.12 mg (or 2 IU) /ml (approximately 0.6 mg/kg). Subjects on each of three testing days received an intraperitoneal (i.p.) injection of either OT or CON. Half of the animal pairs (n=12) received OT (0.012 mg OT /0.1 ml saline) and the remaining pairs (n=12) received an equivalent dose of isotonic saline (0.1 ml). Both animals in a pair received injections of the same treatment. Dosage of OT was derived from a previous study where i.p. administration of OT increased brain levels of OT in the 30 minutes following injection (Neumann et al. 2013).

Phase B: Partner Preference

On the fourth day at 08.30 h, the morning after the third injection, each pair was separated. During this separation period females were kept in their home cage and had olfactory information about their partner. This separation period was decided upon as it meant females had no physical contact with their partner for a couple of hours and would help indicate a clearer preference during the preference test. At 14.00 h the focal female was placed into the central cage of the partner preference setup to allow acclimatisation. At 16.00 h this cage was connected with transparent tubes to the cages with the stimulus females, the focal female was then able to access all three cages. In total, females were in the preference test for 18 hours.

The partner preference setup consisted of two Makrolon Type III cages (width: 23.5 cm, length: 39 cm, height: 15 cm) connected with transparent plastic tubes to a central Makrolon Type II cage (Fig. 1). Each of the Type III cages were bisected laterally with a wire mesh barrier. Stimulus females were placed in the contained halves of the Type III cages. The focal female was placed in the centre cage and had access to all three cages. She was able to interact with stimulus females through the mesh via the use of visual and olfactory cues. The stimulus females could not interact with each other. The focal female was randomly chosen from the

treated pair. Stimulus females were the focal female's previous partner, and a new, unfamiliar, genetically unrelated female, matched in age (age difference: 11.0 ± 2.09 d, mean ± SE) and weight (weight difference: 2.6 ± 0.38 g, mean ± SE); position of stimulus females was randomly assigned.

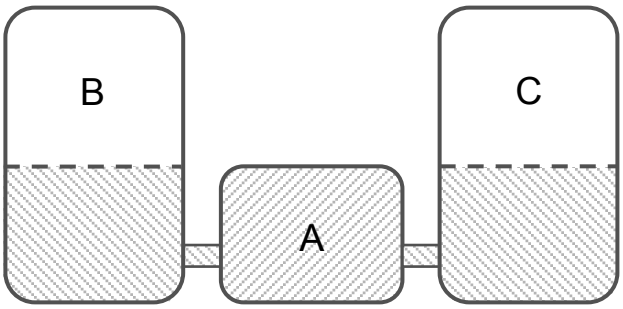

Figure 1. Illustration of the partner preference set up. (A) represents the area accessible to the focal mouse (grey coloured sections). (B) and (C) cages were bisected laterally with a mesh barrier confining them to the white zone. (B) here represents the cohabitation partner of the focal and (C) represents a new unfamiliar, unrelated stimulus female, (B) and (C) were randomly assigned to the left or right side for each new preference test. (A) could interact with both stimulus females via olfactory and visual cues.

Partner Testing

The partner preference began once the focal female had placed at least her two front paws in both sides of the setup (Fig. 1, grey section of (B) and (C)). The side she visited first and the latency to enter each side were recorded. Video recordings were made and a computer app (D.A.T.A, version 1.0.8; Behavior Science.org, LLC) allowed for the scoring of time the focal spent in each cage. Videos were scored blind to treatment group to avoid any bias in the results. Time spent in each cage was recorded for an hour after the test started and then for alternate hours throughout the preference test. Each observation hour began at half past the hour, totalling nine hours of observation per pair. As in previous studies, a social preference was defined as the focal female spending significantly more time with one female over the other (Carter et al. 1992; Insel & Hulihan 1995; Williams et al. 1994; Young & Wang 2004).

Two pairs from phase A were excluded from the partner preference test due to one of the animals breaking through the mesh barrier (both from the control group).

Statistical Analyses

Statistical tests were carried out using R version 3.1.3 (R core team 2015). To analyse time spent (seconds) interacting, either positive or in the same cage and for latencies to meet, we used linear mixed effects models (hereafter: LMM). Occurrence of negative behaviours were analysed with a generalised linear mixed effects model (hereafter: GLMM) using the MASS package in R (Venables & Ripley 2002), and negative binomial was used to correct for over dispersion (Ismail & Jemain 2007). Correcting the occurrence of negative behaviours for time females spent in the same cage yielded the same result as the raw number of behaviours, we therefore used the latter. The proportion of time females spent in the same cage at different time points throughout the day was analysed using a GLMM with binomial error distribution. For all

above mixed effects models, treatment and day were included as fixed effects and, to control for repeated measures, focal pair was included as a random effect.

Amount of time females spent with both stimulus females over the hours of the preference test was compared using a LMM, with hour and treatment as fixed effects and female pair as a random effect. Additionally, we tested total time spent (seconds) with partner and stranger during the first hour with a LMM, again treatment and partner were fixed effects and female pair as a random factor. Here, we included a weight of total time with both females, to account for the difference across pairs. A post hoc test was used to assess within interaction significance using the multcomp package in R (Hothorn et al. 2008), with manually assigned contrasts. To assess latency to enter the first side and to start of the preference test a LM was used, with treatment as the fixed effect. Time females spent in the middle cage and in total with a stimulus female were analysed with a LM, with treatment as a fixed effect.

Model assumptions were assessed for all models visually using diagnostic plots and in the event that they were not fulfilled, data were transformed. Square root transformations were used in LMMs for positive time interacting during cohabitation, time spent with stimulus females in the partner preference test and latency to meet during the cohabitation phase; as well as in LMs for latency to enter first side of the preference set up, and time spent in total with both stimulus females. All linear and mixed effects models, unless otherwise stated, were conducted using the lme4 package in R (Bates et al. 2014). Furthermore, in all models, variance components were estimated using maximum likelihood ("ML") methods and additionally all random effects were kept in the models. We selected the minimal most adequate model through backward stepwise model selection and significance of fixed terms was determined using likelihood ratio tests (Crawley 2007). Table 2 provides means ± standard error of the mean (SE) for time spent in the same cage and together as well as latencies for the cohabitation phase, and time spent in the middle cage and latency to start for the partner preference.

RESULTS

Phase A: Cohabitation

During the cohabitation phase females did not differ significantly in the amount of time spent in the same cage across the three days (LMM: $\chi^2(1) = 2.93$, $p = 0.231$, Table 2), and there was no significant overall effect of treatment (LMM: $\chi^2(1) = 1.02$, $p = 0.313$). Of the time spent in the same cage, there was no significant effect of OT on socio-positive behaviours (LMM: $\chi^2(1) = 0.53$, $p = 0.468$), however, socio-positive behaviours increased significantly across the three treatment days in both groups (LMM: $\chi^2(1) = 13.62$, $p = 0.001$, Fig. 2). Furthermore, time spent in the same cage excluding negative behaviours (i.e. time spent interacting positively plus time in the same cage but not interacting, neutral behaviours) followed the same pattern, a non-significant effect of treatment (LMM: $\chi^2(1) = 0.68$, $p = 0.411$) and a significant increase across the three days (LMM: $\chi^2(1) = 11.67$, $p = 0.003$, Table 2).

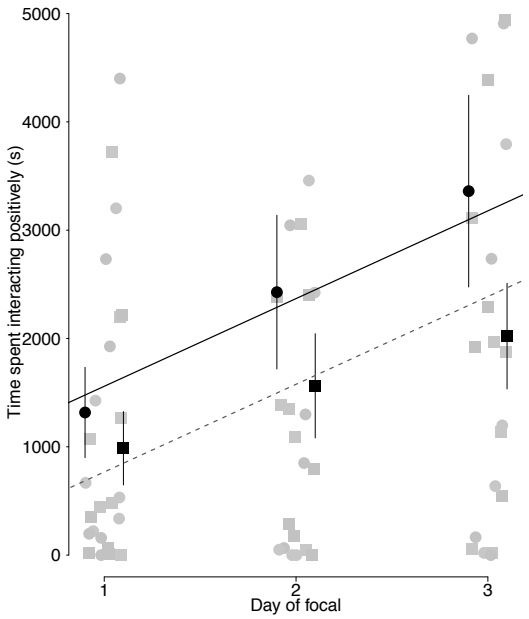

Figure 2. **Time spent interacting positively across the three treatment days**. There was a significant increase in positive interactions across the three treatment days, in both treatments ($p < 0.01$). Dark symbols illustrate mean ± SE for positive interactions for each treatment per day, grey symbols indicate the total time per pair per day (solid line (circles): OT, Dashed line (squares): CON).

Occurrence of socio-negative behaviours was generally rare, even during the first day, and did not differ significantly across the two treatment groups (GLMM: $\chi^2(1) = 0.15$, $p = 0.701$, Fig. 3), but there was a significant decline in the number of negative interactions across the three days (GLMM: $\chi^2(1) = 54.17$, $p < 0.001$). Treatment had no significant effect on the latency of the animals to meet (LMM: $\chi^2(1) = 0.17$, $p = 0.679$, latency to first interaction on the first and subsequent days, Table 2) but latency to meet decreased significantly across the three days of the experiment (LMM: $\chi^2(1) = 28.77$, $p < 0.001$). Furthermore, there was no significant effect of treatment on the proportion of time females spent in the same cage at the different time points measured throughout the night and day (GLMM: $\chi^2(1) = 0.03$, $p = 0.870$). Females were found in the same cage significantly more often than in separate cages across the three days (GLMM: $\chi^2(1) = 7.85$, $p = 0.005$).

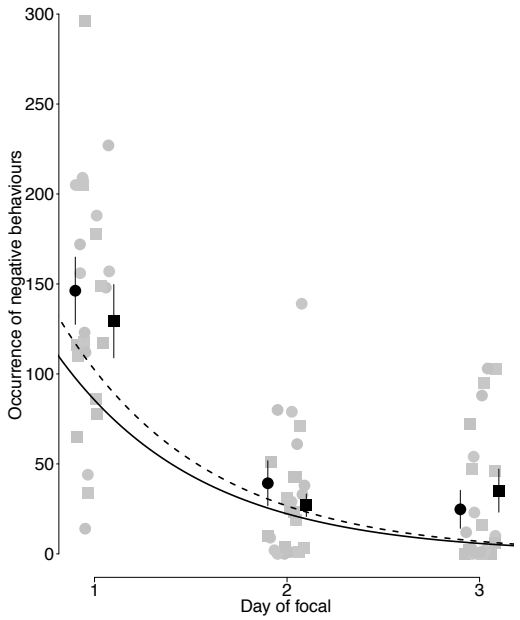

Figure 3. **Occurrence of negative behaviours across the three treatment days**. There was a significant decrease in negative behaviours across the three treatment days, in both treatments ($p < 0.01$). Mean ± SE denoted by dark symbols for each treatment per day, grey symbols indicate the total occurrence per pair per treatment (solid line (circles): OT. Dashed line (squares): CON)

Phase B: Partner Preference

Over the nine hours analysed for the preference test the time females spent with the stimulus females differed significantly (LMM: χ^2 (8) = 16.02, p = 0.042), regardless of treatment (LMM: χ^2 (1) = 0.39, p = 0.531). Whereby, time spent with stimulus females decreased during the 2nd and 3rd hour tested but was otherwise similar in the remaining hours. Suggesting a 9-hour observation period may not be necessary for determining preference in mice using this setup. Given this observation, we focused on the preferences displayed in the first hour, when time spent with females was above the overall average, and 18 of 22 females were in the same cage as their cohabitation partner for 50% or more of the time with a stimulus female (OT: 9/12, CON: 9/10).

During this first hour of the preference test there was a significant interaction between treatment and partner, whereby CON females spent significantly more time with their partner than OT females (LMM: χ^2 (1) = 6.29, p = 0.012, Fig. 4). Post hoc tests revealed that OT females showed no significant difference in the amount of time spent with either stimulus female (LMM: z = 1.49, p = 0.327, Fig. 4). However, CON females spent significantly more time with their partner than the stranger (LMM: z = 5.55 $p < 0.001$, Fig. 4). Time spent in the middle cage during this hour did not differ significantly across the two treatments (LM: $F_{1, 20}$ = 0.01, p = 0.941, Table 2).

Table 2. Mean and standard error of the mean (SE) for analysis where no figure has been provided. Times given by treatment and day, except in the case of the partner preference where means are given only by treatment.

	Cohabitation						Partner preference			
Beh	Same cage[a] (s)		Together[b] (s)		Latency to meet (s)		Latency to start (s)		Middle cage (s)	
Trt	OT	CON	OT	CON	OT	CON	OT	CON	OT	CON
Day										
1	4834.0±391.4	4664.7±404.5	3577.2±393.7	3398.3±397.1	362.0±109.3	378.2±72.8	972.3±213.9	1171.7±451.8	1888.0±382.7	1849.6±326.5
2	5631.6±577.8	4393.3±604.7	5112.9±666.5	4053.4±615.9	125.4±38.4	75.3±11.8				
3	5658.1±724.1	5513.6±546.8	5340.4±769.2	5186.1±509.2	136.7±55.7	74.1±21.2				

Trt, treatment; Beh, behaviour.

[a]Same cage = time females spent in the same cage as each other regardless of interaction type.

[b]Together = the time females spent together in the same cage minus any time interacting negatively.

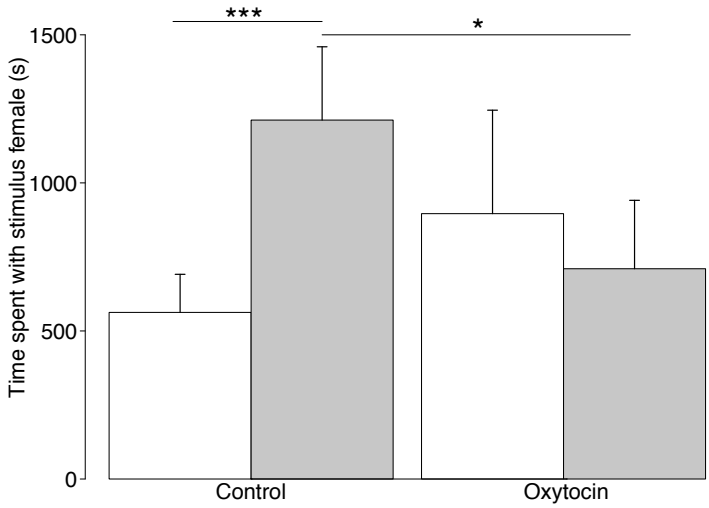

Figure 4. Partner preference test. Time females spent (in the first hour), mean ± SE, in the cage with the partner (grey) and with the stranger (white) for each treatment, * < 0.05, *** < 0.001, ns = when no asterisks indicated.

OT and CON females did not differ significantly in the total time spent with another female regardless of whether she was the partner or stranger (LM: $F_{1,20} = 0.33$, $p = 0.572$). Treatment did not significantly affect the latency to enter the first side of the preference setup (LM: $F_{1,20} = 1.42$, $p = 0.248$) or the latency to the start of the partner preference (LM: $F_{1,20} = 0.18$, $p = 0.678$).

DISCUSSION

Female house mice cooperatively raise communal litters and form partnerships that have been shown to increase reproductive success (König 1994). In the past studies have suggested that time spent in close association can be used as a measure for social bonds among individuals (Silk et al. 2003; Weidt et al. 2008; Cameron et al. 2009; Schülke et al. 2010). Therefore, it could be assumed that female mice establish social bonds prior to communally nursing through spending time interacting positively with one another. Our study investigated whether injecting oxytocin (OT) could play a role in the facilitation of initial behaviours between unfamiliar females and influence female preference for a cohabitation partner. Contrary to our expectations, exogenous OT administration did not alter initial prosocial behaviours between two unfamiliar, unrelated and non-reproducing females, and interfered in the formation of a preference for a familiar female.

Socio-positive behaviours significantly increased over the three days in both treatments, suggesting that females generally became more affiliative and tolerant of one another. However, in the subsequent preference test, it was only CON females who spent significantly more time in the same cage with the partner than the stranger, which implied they had formed a preference for their partner. Time spent close to or in the same cage as a conspecific is commonly interpreted as a preference, and has been used in previous studies (Carter et al. 1992; Insel & Hulihan 1995; Williams et al. 1994; Young & Wang 2004). Despite OT females exhibiting the same pattern in their behaviours as CON females during the three days of cohabitation, they did not demonstrate a preference for their cohabitation partner when given a choice of partners.

Instead they spent similar amounts of time with both stimulus females, suggesting that OT interfered with the formation of a partner preference.

Treatment Effects on Female Behaviour

OT has been suggested to promote sociability and motivate individuals to affiliate more generally (Campbell 2008). This has been demonstrated in studies where animals treated with OT showed increased social contact (Witt et al. 1990; Carter et al. 1992), and increased exploratory behaviours (Dharmadhikari et al. 1997). While a lot of attention has been given to the positive effects of OT, there are also negative ones (Beery 2015), and OT has been found to enhance both positive and negative perceptions (De Dreu et al. 2012; Crockford et al. 2014). Furthermore, the influence of OT has been related to inter-individual competition (Radke & de Bruijn 2012), and when information related to a partner is inaccessible OT can reduce cooperation (Declerck et al. 2010). OT manipulation was demonstrated to prevent stress induced social avoidance in rats, and facilitated a social preference for novel conspecifics in both rats and mice (Lukas et al. 2011). However, Peñagarikano et al. (2015) found that during a social interaction test, pairs of wild type mice treated with OT did not differ in time spent interacting socially on first encounter with a stranger, when compared against control treated pairs. These findings support those of the current study, as OT did not influence time spent with a stranger on first encounter or facilitate a preference for the previous partner. Additionally, in female meadow voles OT increased time spent huddling with a preferred partner but was not required for social preference formation, as control females formed a preference regardless of treatment (Beery & Zucker 2010). Results from our study support such findings, as OT did not increase social interactions between pairs of new, unfamiliar individuals, beyond those that would be expected naturally. Taken together, these results, combined with findings from the current study, support the idea that elevated endogenous OT is not a facilitator in initial social interactions among female house mice. Consequently, OT does not seem to affect the process of females becoming familiar, yet when compared against the CON, it hinders the formation of a preference.

Some alternative explanations for the OT females in the current study spending similar amounts of time with both stimulus females could be that they were showing increased exploratory behaviour (Dharmadhikari et al. 1997; Windle et al. 1997; Lukas et al. 2011). Windle et al. (1997) found that mildly stressed rats treated with OT spent a higher proportion of time in the open arms of an elevated maze. Furthermore, Uvnäs-Moberg et al. (1994) found high doses of peripherally administered OT to increase the amount of time individuals spent away from the perceived security of a boundary wall. Additionally, OT has been suggested to have anxiolytic effects on behavioural systems, which could moderate the anxiety response to stress (Windle et al. 1997; Smith and Wang 2014), and injection with OT was demonstrated to have anti-stress effects comparable to those produced by positive social stimuli (Uvnäs-Moberg 1998). This could suggest that the focal female considered the unfamiliar female less aversive and easier to approach during the preference test. However, in the current study we did not explicitly test for exploratory behaviour or a stress response, therefore future research could investigate such hypotheses. OT has also been demonstrated to be an essential peptide for facilitating familiarity recognition and the ability to distinguish familiar from unfamiliar conspecifics (Choleris et al. 2003, 2009). OT in our study may have facilitated discrimination of familiar from unfamiliar individuals, and could even have enhanced the focal female's response to the unfamiliar partner.

Past studies have shown that some behavioural effects of OT could be linked to the activation of vasopressin receptors (reviewed in: Freeman and Young 2016), such as the V1a receptor (Busnelli et al. 2013; Bowen and McGregor 2014). In the current study, this could suggest that OT interfered with preference formation through binding at the V1a receptors. Given that studies using similar doses and routes of administration have lead to contrary findings (Cushing and Carter 2000; Bowen and McGregor 2014), we cannot discard the possibility that OT administration in our study also acted through vasopressin receptors.

The Importance of Social Preferences

It has been shown that female house mice preferentially allonurse with kin or familiar females, and in doing so maximise their reproductive success (König 1994). However, females also develop non-random preferences for social partners when kept in groups of unrelated females, with roughly three-quarters of females showing significant associations with at least one other female (Weidt et al. 2008). Furthermore, Weidt et al. (2008) demonstrated that female house mice have greater reproductive success with preferred over un-preferred partners when both are unrelated. Despite females, in the current experiment, not getting the opportunity to choose their initial partner, we found a steady increase in positive interactions across the three days, suggesting females became more affiliated. Additionally, OT females behaved similarly to CON females during cohabitation, but when presented with a novel, unknown female, they became choosier, supporting the idea that perhaps OT increased social approach behaviour (Lim & Young 2006) or increased salience of social stimuli (Young & Barrett 2015). Further research would be required to disentangle such ideas, as well as determine whether females would go on to form a communal nest together, and whether OT can play a role in this.

Route of Administration

When interpreting our results it must be considered that the peripheral administration of OT may not have crossed the blood brain barrier. Findings from previous studies, however, suggest small quantities may do so. Dosage of OT for this study was derived from previous research, in particular the findings of Neumann et al. (2013) who found increased OT in brain dialysates 30 minutes post intraperitoneal injection in mice. Their findings provided initial evidence for the uptake of peripherally administered synthetic OT into the brain, although the routes of entry were unknown (Neumann et al. 2013). These results are supported by additional studies that used peripheral administration of OT to assess its behavioural effects. Mooney et al. (2014) found increased huddling behaviour and time in close proximity to conspecifics in the naked mole rat. Meerkats injected subcutaneously with OT demonstrated increased cooperative behaviours such as digging and pup feeding as well as decreased initiation of aggression (Madden & Clutton-Brock 2011); and peripheral OT administration inhibited infanticide in female house mice (McCarthy 1990). Additionally, intraperitoneal injection of OT significantly increased time in social contact with a novel partner in Cntnap2 mutant mice compared against the vehicle control (Peñagarikano et al. 2015). Cntnap2 mice have social behaviour deficits linked to autism and reduced expression of OT within their neurons, therefore this increase in social behaviour suggests OT may have crossed the blood brain barrier (Peñagarikano et al. 2015). Therefore, these past studies among others suggest peripheral administration of OT to be justifiable as a method of manipulation and to assess its effects on social behaviours.

Conclusions

Our results demonstrate that unfamiliar female house mice naturally became more affiliative towards one another over time (as demonstrated by the CON), and that this process appears not to be affected by treatment with OT. However, our results also suggest that injecting exogenous OT prevented the formation of a preference for a cohabitation partner. Additional research should be carried out to investigate this further, by measurement of peripheral and central OT levels, or testing whether behaviours are reversed when an OT antagonist is introduced. These results contribute to our growing knowledge of OT and its variable influence on social behaviour; they support findings that suggest its effects can be very context and perhaps species specific (Insel & Young 2001; Campbell 2008; Donaldson & Young 2008; Radke & de Bruijn 2012). Additionally, they support the idea that the role of OT can be influenced by many factors including other hormonal effects (discussed in: Campbell 2008) and contact with a known or preferred partner (Beery & Zucker 2010; Crockford et al. 2013). Lim and Young (2006) discuss how attachment bonds can be both "selective and enduring" between individuals and social bonds require a combination of many processes. Consequently, with regard to social partner preferences among female house mice, there remains plenty of scope to discover more about their social behaviour and factors that may influence choice of social partner.

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CHAPTER 4

Oxytocin Administration During Early Pair Formation Delays Communal Nursing in Female House Mice

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Oxytocin Administration During Early Pair Formation Delays Communal Nursing in Female House Mice

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ABSTRACT

Oxytocin manipulation has been implicated in the facilitation of social and cooperative behaviours, either through increasing positive and cooperative social interactions, or facilitating bond formation. Here we aimed to determine whether peripheral administration of oxytocin would affect the propensity of unrelated female house mice, *Mus musculus domesticus*, to cooperate. To investigate this, we used female house mice, with their cooperative ability to communally nurse offspring. Pairs of unfamiliar females received intraperitoneal injections of oxytocin over a 3-day cohabitation period. Following this initial phase, a male was introduced and allowed mate with the females. We monitored how long it took females to establish and successfully cooperate in the raising of a communal litter. Oxytocin did not affect the females' ability to reproduce. However, oxytocin-treated females took significantly longer to establish a successful communal litter (with pups of both partners being weaned) than saline-treated control females. This delay in communal nursing was due to higher pup mortality and loss of first-born litters in the oxytocin group during their first reproductive event. We conclude that administration of exogenous oxytocin during the early stages of the female relationship delayed the tendency of female house mice to affiliate and cooperate in rearing a communal litter. Our findings contribute to the growing field of oxytocin-based studies and sheds light on the potential long term effects of oxytocin during early pairwise social interactions.

INTRODUCTION

The oxytocinergic system has been implicated in the facilitation of a variety of social and cooperative behaviours as well as the suppression of the stress response (Carter, Williams, Witt, & Insel, 1992; Carter & Wilkinson, 2015; Crockford, Deschner, Ziegler, & Wittig, 2014; Crockford et al., 2013; McCarthy, 1990; Mooney, Douglas, & Holmes, 2014; Popik, Vetulani, & van Ree, 1992; Smith, Ågmo, Birnie, & French, 2010; Witt, Carter, & Walton, 1990; Wittig et al., 2014). Over the last couple of decades evidence for the role of the peptide hormone oxytocin (OT) in the facilitation of social bonding, affiliative and cooperative behaviours has accumulated in a range of species (Anacker & Beery, 2013; Beery & Zucker, 2010; Carter & Wilkinson, 2015; Crockford et al., 2013; Mooney et al., 2014; Wittig et al., 2014). While central administration of OT was thought to be essential, current evidence indicates that peripheral administration also induces behavioural effects (McCarthy, 1990; McCarthy, Bare, & vom Saal, 1986; Mooney et al., 2014; Popik et al., 1992; Smith et al., 2010), which has simplified manipulations in less traditional species (such as primates and humans). For example, virgin and pregnant female house mice, *Mus musculus domesticus*, demonstrated decreased infanticidal behaviour towards pups placed in their homecage after subcutaneous injection with OT (McCarthy et al., 1986); and female prairie voles, *Microtus ochrogaster*, demonstrated a preference for a previous male cohabitation partner after receiving peripheral pulses of OT (Cushing & Carter, 2000). Additionally, huddling and partner-seeking behaviour were facilitated by intranasal OT in male and female marmosets, *Callithrix penicillata* (Smith et al., 2010). In the context of cooperation, subcutaneously injected OT increased a range of cooperative behaviours including pup feeding, digging and guarding behaviours in wild meerkats, *Suricata suricatta* (Madden & Clutton-Brock, 2011), and intranasal administration of OT increased time spent allogrooming in the common vampire bat, *Desmodus rotundus* (Carter & Wilkinson, 2015). Furthermore, elevated OT levels were found in chimpanzee, *Pan troglodytes*, urine following socio-positive or cooperative interactions, such as food sharing with conspecifics (Wittig et al., 2014) and grooming with a preferred partner (Crockford et al., 2013).

To summarize, many results from studies investigating the effects of OT provide evidence that OT increases positive perceptions and social motivation (Crockford et al., 2014; Cushing & Carter, 2000; Madden & Clutton-Brock, 2011; McCarthy, 1990; Mooney et al., 2014; Smith et al., 2010). However, despite its ability to amplify pre-existing positive social perceptions it can also intensify negative ones (Beery, 2015; Crockford et al., 2014; De Dreu, Shalvi, Greer, van Kleef, & Handgraaf, 2012) and be context and partner specific (Bartz, Zaki, Bolger, & Ochsner, 2011; Beery, 2015; Campbell, 2008; Crockford et al., 2013; Cushing & Carter, 2000; Cushing, Martin, Young, & Carter, 2001; Declerck, Boone, & Kiyonari, 2010; Wittig et al., 2014). Negative effects of OT have been found in capuchin monkeys, *Cebus apella*, that demonstrated reduced cooperative food-sharing behaviour after receiving intranasal OT (Brosnan et al., 2015), and female house mice exhibited no preference for a previous cohabitation partner after intraperitoneal injection of OT (Harrison, Lopes, & König, 2016). Furthermore, humans who received intranasal OT demonstrated increased envy (Shamay-Tsoory et al., 2009), decreased cooperation when social information was lacking (Declerck et al., 2010), and in a study where subjects were confronted with an unreliable partner, OT lost its trust-enhancing effects (Mikolajczak et al., 2010). Whether through its positive or negative actions, OT is a prime candidate for having a role in intra-sexual cooperation. In particular, we

were interested in whether OT plays a role in the ability of females to cooperate through communal offspring care, thus affecting individual fitness.

To explore such notions, female house mice offer an ideal study system as they have been shown to cooperate by nursing communally in a range of different settings including the laboratory, semi-natural and wild environments (Manning, Wakeland, & Potts, 1992; Weidt, Lindholm, & König, 2014; Wilkinson & Baker, 1988). In mice, communal nursing is determined when two or more females pool their litters in a single nest and raise them together. We call these pooled litters a communal litter. Once litters are pooled females do not distinguish between their own and other offspring (Hayes, 2000; König, 1989; 1994b; Packer, Lewis, & Pusey, 1992). Therefore, females will cooperate in the care of their young by nursing all offspring in a nest indiscriminately (Ferrari, Lindholm, & König, 2015; König, 1989; 1994a). Female mice often form such egalitarian relationships with familiar sisters leading to increased lifetime reproductive success for both females (König, 1994a). However, females also form individual preferences for unrelated individuals when kept in laboratory enclosures (Weidt, Hofmann, & König, 2008), where they will communally nurse even when given the option to rear litters alone. Communal nursing provides many benefits for females such as allowing them to wean more offspring in their lifetime (König, 1997), and provides the opportunity to spend more time foraging, as their partner attends the litters in their absence (Auclair, König, Ferrari, Perony, & Lindholm, 2014). However, free-living females will also nurse their pups solitarily (raise pups alone) despite having potential communal nursing options available to them (Weidt et al., 2014). This suggests that there is an element of choice and additional factors involved when deciding whether or not to nurse communally, and with whom.

In this study we aimed to determine whether OT influenced a pairs ability to cooperate by communal nursing. To do so we experimentally increased peripheral OT in pairs of unrelated, unfamiliar female house mice over 3 days before introducing a male. In contrast to familiar sisters, unrelated unfamiliar females vary in their propensity to nurse communally with a randomly assigned female partner in an experimental situation (König, 1994b; Palanza, Dellaseta, Ferrari, & Parmigiani, 2005). If OT has a positive effect on female social relationships that results in reproductive cooperation, we would expect to see this when treating pairs of unrelated, previously unfamiliar females. After the initial treatment, females were allowed to reproduce and we monitored how long it took them to establish and successfully wean a communal litter.

We recently found evidence suggesting that OT treatment prevents or lessens the formation of a preference for a cohabitation partner in female house mice (Harrison et al., 2016). Given these current findings and those of studies in which OT was found to reduce cooperation, we predicted that females in the OT treatment would take longer to nurse communally than control females, indicating a decreased propensity to cooperate.

METHODS

We used wild derived, laboratory born F1 to F3 descendants of house mice originating from a barn population near Zurich, Switzerland, as described in König and Lindholm (2012). Weaning occurred at day 23 and subsequently animals were kept in same-sex sibling groups until 11-14 weeks of age, when females were sexually mature and the OT system was well developed (in rodents, the OT system is already developed at weaning, Yamamoto et al., 2004). Cages contained standard animal bedding (Lignocel hygienic animal bedding, JRS), with cardboard and tissue provided *ad libitum* for bedding and shelter. Mice were kept under a

constant light:dark cycle of 14:10 hours (lights on at 0530 hours CET, with a half hour dawn and dusk phase at the beginning and end of the light phase), at a temperature of 22-24°C and humidity of 50-55%. At all stages of the experiment, food (laboratory animal diet for mice, Provimi Kliba SA, Kaiseraugst, Switzerland) and water were provided *ad libitum*. Animal use and experimental design were approved by the Veterinary Office Zurich, Switzerland (Kantonales Veterinäramt, Zurich, no. 34/2014).

Experimental Procedures

Pairs of virgin unfamiliar, unrelated females (pairs did not share the same parents) were randomly assigned to one of two treatment groups, oxytocin (OT, $N = 14$ pairs) or saline control (CON, $N = 14$ pairs), and both females in a pair received the same treatment. Female pairs were matched, as best as possible, in age (age difference: 5.0 ± 0.6 days, mean ± SE) and weight (weight difference: 2.1 ± 0.3 g, mean ± SE). For identification females were marked with different ear punches.

At the beginning of the experiment, each female was housed in a separate Makrolon Type II cage (18 x 24 cm and 14 cm high) for 30 minutes, equipped with a transparent plastic tube (4 cm diameter) that would allow access to the partner female's cage. Entry to the neighbouring cage was initially prevented with a removable barrier. Both females in the pair were then given an intraperitoneal injection of their treatment, on each of three consecutive days between 1600 and 1800 hours. Following each injection females were allowed a 15 min recovery period in their own cage before the barrier was removed allowing each female of a pair access to both cages and to freely interact. On the fourth day, the morning after the third injection day, an unrelated male (not sharing a parent with either of the two females) was introduced. The male's home cage, also Type II, was connected by a transparent tube to the two female cages. The two females and the male thereafter had access to all three cages and we refer to the three mice sharing a cage system as a group.

Throughout the experiment, cages were checked daily for any signs of aggression among the mice; in the event of excessive aggression, resulting in wounds, groups were separated. Once a day we determined whether females were resting together in a nest (side-by-side contact, a measure of affiliation), starting from the introduction of the male until at least one litter was weaned. Nineteen days after the introduction of the male, cages were additionally checked daily for birth of litters (house mice have a gestation period of 19-21 days, König, 2012). Assignment of a litter to a female was based on visual inspection (lack of body swelling of a previously pregnant female) and a decrease in female body weight. To avoid any bias groups were checked blind to treatment group. Once a litter was born, females and pups were weighed on set days following birth until weaning [day 1 (birth), 5 (pups only), 9, 13, 17 and 23] when the pups were removed. Additional features were determined including litter size and sex ratio. These measurements allowed us to determine any pup mortality and monitor condition of the mice throughout. In a previous experiment, female pairs received identical OT treatment and socio-positive and socio-negative behaviours were monitored in a series of focal observations over a 3-day cohabitation period (Harrison et al., 2016). Results from that study indicated no significant difference in behaviours between OT and CON treated female pairs; therefore, in the current study, these detailed behavioural observations were not made.

We determined a litter to be communal in this study when the second female gave birth within 16 days of the first female and pups were raised in a single nest (the same definition used by Ferrari, Lindholm & König, 2016; König, 1994a). Day 16 was chosen as weaning

commences 1 day later when pups begin to eat solid food and reduce milk consumption (König & Markl, 1987). Since offspring of 17 days or older have only a small influence on female investment we no longer considered this as a communal litter (Ferrari et al., 2016). A successful communal litter was one in which at least one pup from each litter was weaned (day 23), and pups had been raised in the same nest. An unsuccessful communal litter was one in which a second litter was born within 16 days of the first, but no pups or only pups from one of the litters survived until weaning. Litters could be distinguished based on differences in pup development. A solitary litter was one in which pups were born in the absence of another un-weaned litter and when no other litter was born within 16 days.

Once each pair had successfully weaned a communal litter the experiment was complete. However, if a pair did not successfully wean a communal litter within 3 months from the date the male was introduced, the experiment was terminated and the pair considered unsuccessful. To calculate the latency until formation of the first communal litter we calculated the number of days, starting from the day the male was introduced, until the second female gave birth (only when the first female had given birth no more than 16 days before and the pups were pooled in the same nest).

To assess the effect of treatment on birth order within a communal litter we classified litters into three birth order categories (the order females gave birth): first-born was the first-born litter in a communal litter (no other litter born in the previous 16 days); second-born was a litter born within 16 days of the first-born litter and if no other litter was born within 16 days thereafter; and middle-born (when necessary) was a second litter born within 16 days of the first litter (with at least one pup surviving until weaning) and another litter was born within 16 days of the second-born litter.

Peptide and Doses

Synthetic oxytocin (Product: O4375-250IU, Sigma Aldrich Co., Munich, Germany) was dissolved in sterile saline (0.9% NaCl, Bichsel, Unterseen, Switzerland) to give a concentration of 0.12 (or 2 IU) mg/ml. Subjects on each of 3 test days received an intraperitoneal injection of either OT or CON. Half of the animal pairs (N = 14) received OT (0.012 mg OT/0.1 ml saline: approximately 0.6 mg/kg) and the remaining pairs (N = 14) received an equivalent dose of isotonic saline (0.1 ml). Both females in a pair received the same treatment. Dosage of OT was derived from a study in which a peripheral injection of OT led to increased circulating OT for up to 2 h and a peak in brain dialysates 30 min after administration (Neumann, Maloumby, Beiderbeck, Lukas, & Landgraf, 2013). The same dosage and/or route of administration have also been used in other studies with house mice and other rodents with effects on behaviour (Harrison et al., 2016; Mooney et al., 2014; Peñagarikano et al., 2015).

Statistical Analysis

Statistical tests were carried out using R version 3.1.3 (R Core Team, 2015). Linear models (LM) were used unless the data required random effects to control for repeated measures (more than one litter per female or to identify the pair). In these cases, linear mixed models (LMM) and/or generalized linear mixed models (GLMM) were required and analysed using the 'lme4' package in R (Bates, Maechler, Bolker, & Walker, 2014). Where appropriate, we assessed for normality of model assumptions visually using diagnostic plots and in the event that they were not fulfilled data were transformed (Table 1). Models using a binomial error

distribution were tested for overdispersion. Model selection was carried out to find the best model using the full model and comparing it to all lower models using the model selection function in the 'MuMIn' package (Bartoń, 2015). Models were ranked by corrected Akaike information criterion (AICc) values and the most adequate model was the one with the lowest AICc value. If two or more models fell within two delta AICc of each other they were considered equal and therefore the model with the lowest degrees of freedom was chosen.

Table 1 illustrates all models used to analyse the effect of treatment on the females' propensity to nurse communally, as well as on litter sizes and pup mortality. Response variables and fixed effects are provided for the full models and most adequate models, where required random effects are also stated. To determine the significance of the fixed effects for the most adequate model only we used likelihood ratio tests for GLMM and LMM (Crawley, 2007), and *F* tests for LMs. Fixed effects that were not included in the most adequate model were defined as non-significant and therefore no *P* values are provided. In the case of pup survival for the first communal litter, post-hoc analysis was carried out on the interaction term to better understand within-interaction effects, using manually assigned contrasts in the 'multcomp' package in R (Hothorn, Bretz, & Westfall, 2008). Additionally, we used a chi-squared test (effect size given as Φ) to determine whether there was a difference in the total number of communal litters born by treatment.

Table 1. Summary of all models used in the data analysis

Type of model	Response variable	Fixed effects		Random effects
		Full model	Best model	
OT effects on the propensity to nurse communally				
1. LM (log)	Latency to birth of successful CL	trt * wdf + adf	trt	
2. LM	Latency to first CL born	trt	Intercept only	
Effect of treatment on litter attributes				
3. LMM	Litter size at birth	trt * litnum	litnum	PairID/FemID
4. LMM	Litter size at weaning	trt * litnum	litnum	PairID/FemID
5. LMM	Weaning weight of pup	trt * ord + lsw + adp	Intercept only	PairID/FemID LitID
OT effect on pup survival				
6.GLMM (binomial)	Proportion of pups survived	trt * ord + adp	ord	PairID/FemID
7.GLMM(binomial)§	Proportion of pups survived§	trt * ord + adp	trt * ord	PairID
8.GLMM (Poisson)	Number of pups killed#	lsb + lsp + trt	Intercept only	PairID
OT effect on female resting/affiliative behaviour				
9. GLMM (Poisson)	Days in side-by-side contact	trt * puppres	puppres	PairID

List of abbreviations: CL = communal litter. Successful CL = at least one pup from each litter survived until weaning. trt = Treatment (OT or CON). adf = age difference between the two females. wdf = weight difference between the two females at the beginning of the experiment. litnum = a female's litter number, whether it was her first, second etc. ord = birth order in a CL (first-, second- or middle-born litter – not included in §). adp = age difference between the two litters in the CL, $\left[\sqrt{(age\ of\ focal\ litter - age\ of\ other\ litter)^2}\right]$. lsw = litter size at weaning. lsb = litter size at birth of first-born litter born in a CL. lsp = litter size of the second female to give birth in a CL. puppres = whether or not pups were present in the cage setup. FemID = ID of the female that gave birth. PairID = the pair that the females belonged to. LitID = the litter the pup belonged to.
§ Pup survival for first CL only (successful or unsuccessful).
# First-born litter in the CL only (successful and unsuccessful CL).

RESULTS

Initially 28 pairs (N = 56 females) were included in the experiment, 14 pairs per treatment. Of these 28 pairs, four were excluded due to aggressive males or incompatible females before reproduction commenced (two pairs from each treatment). Aggression, due to incompatibility, between unfamiliar females leading to separation is not an unusual occurrence (König, 1994a; Weidt et al., 2008). Therefore, 24 pairs (12 per treatment) were included in the final analysis. Of these, three pairs were separated after birth of the first litters due to male aggression towards one or both females: therefore, as these separations were linked to the male and not the incompatibility of the females, for some analyses these pairs were not included (excluded from models 1 and 6, Table 1 and Chi squared test).

OT Effect on the Propensity to Nurse Communally

In total 68 litters were born to the 24 pairs of females (12 per treatment) of which 32 litters were raised successfully and weaned as communal, nine by CON pairs (18 litters) and seven by OT pairs (14 litters). The remaining three CON and five OT pairs failed to successfully raise a communal litter within 3 months (Table 2). In total, there were 17 unsuccessful communal litters; in three cases both litters were lost completely and in 14 cases only the first-born litter was lost completely. Three litters (two OT, one CON) were raised solitarily (where no other litter was born within 16 days) and one litter was born solitary but not weaned. In two pairs there were overlapping litters (middle-born); these litters were then counted twice, first as an unsuccessful communal litter (first-born: A1; middle-born: A2) and then as a successful communal litter (middle-born: A2; second-born: A3). This means the number of litters accounted for was 70 but only 68 separate litters were born. On one occasion both females in a pair (CON) gave birth on the same day. As this precluded determination of birth order, we allocated half the pups to each female; all of these pups were found dead (N = 14 pups).

Table 2: Information on all the litters born by treatment

	OT	CON
First CL successful	1	7
Number of first litters that failed[a]	9	4
Number of second litters that failed[a]	0	3
Number of pairs with a successful CL	7	9
Number of pairs that had more than one attempt at forming a successful CL	6	3
Total number of pairs	12	12
Total number of CL	18	15
Number of unsuccessful CL[b]	11	6

[a] First and second litter born to each pair; the number that suffered complete loss of pups in the first CL attempt

[b] Values out of the total communal litters (CL); otherwise given as the total for the 12 pairs by treatment.

Female pairs that had received OT took significantly longer to successfully rear a communal litter than CON pairs (LM: $F_{1, 14} = 8.72$, $P = 0.010$: Fig.1); the latency (mean ± SE) in days from introduction of the male to formation of a successful communal litter was 51.71 ± 5.32 days for OT females and 30.67 ± 4.85 days for CON females. However, there was no significant difference between treatments in the latency until birth of the first communal litter, whether successful or unsuccessful (LM: $F_{1, 19} = 1.75$, $P = 0.202$; Fig. 1); the mean ± SE latency for OT was 29.64 ± 3.94 days and for CON 23.90 ± 0.32 days. There was also no significant difference in the number of communal litters born, successful and unsuccessful combined (chi-squared test: $\chi_1^2 = 1.27$, $P = 0.261$, $\Phi = 0.21$, $N = 30$). There was no significant difference between treatments (GLMM: $\chi_1^2 = 1.03$, $P = 0.320$) in the number of days that females were observed resting together; however, in the presence of pups, pairs spent significantly less time resting in side-by-side contact than during the time before a litter was born in both treatments (GLMM: $\chi_1^2 = 4.15$, $P = 0.042$). Females never established separate sleeping nests or had litters in separate nests

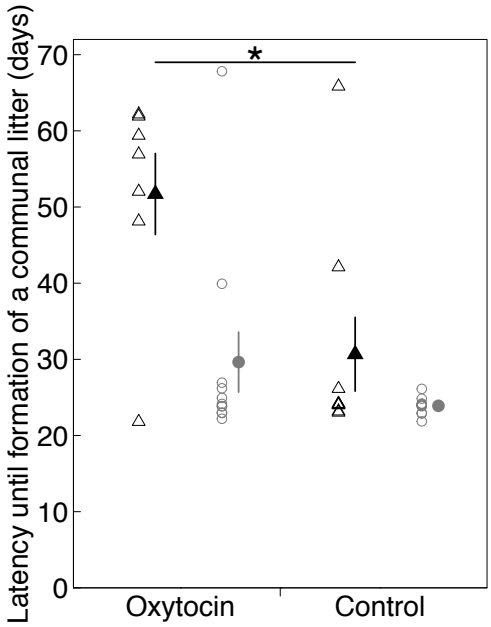

Figure 1. The latency until formation of the first successful communal litter (black, triangles), and the latency until the first communal litter was born whether successful or unsuccessful (grey, circles). Shown for both treatments (mean ± SE), * $P < 0.05$.

OT Effect on Pup Survival

From the 68 litters born there were 419 pups, of which 240 (57.3%) survived until weaning. Pup survival overall was 59.9% in the OT group and 53.3% in the CON group. First-born litters experienced complete or partial loss of pups in 34 of 37 (91.9%) litters compared to only eight of 27 (29.6%) second-born litters. In the OT group 10 of 18 (55.6%) first-born litters in a communal litter suffered a complete loss of pups compared to six of 15 (40.0%) first-born litters in the CON group. Furthermore, 58.3% of first attempts of communally nursed litters were successful in CON pairs compared to 8.3% of OT pairs (Table 2). We recorded litter loss as infanticide when pups were found with their head bitten off or had wounds to the neck region (66.2% of litters suffered complete or partial loss of pups). Although female house mice have

been shown to kill pups of another female when heavily pregnant (Ferrari et al., 2016; König, 1994a; 1994b; McCarthy et al., 1986), it is also known that under certain circumstances females will kill their own pups (Poley, 1977; König, 1989b). We thus could not ascertain the identity of the female(s) performing the infanticide.

For all litters born there was no overall effect of treatment on pup survival but there was a significant effect of birth order (GLMM: $\chi^2_2 = 22.48$, $P < 0.001$), where pups from first-born litters had a lower probability of survival than pups from second- and middle-born litters. There was no significant effect of age difference between the two litters. Additionally, we analysed pup survival in the first communal litter attempt only (this included successful and unsuccessful communal litters) for each pair, to assess whether this had an influence on the latency until formation of the first successful communal litter. We found a significant interaction between treatment and birth order (GLMM: $\chi^2_1 = 4.44$, $P = 0.035$), and post hoc analysis revealed that in the OT group only pups from the first-born litter had a significantly lower survival probability than pups from the second-born litter ($z = 4.15$, $P < 0.001$: Fig. 2). There was no significant difference in survival between first-born and second-born litters of the CON group ($z = 2.12$, $P = 0.067$). However, there was a tendency for lower survival in first-born CON litters, this was in accordance with the overall effect of birth order in model 6 (see Table 1).

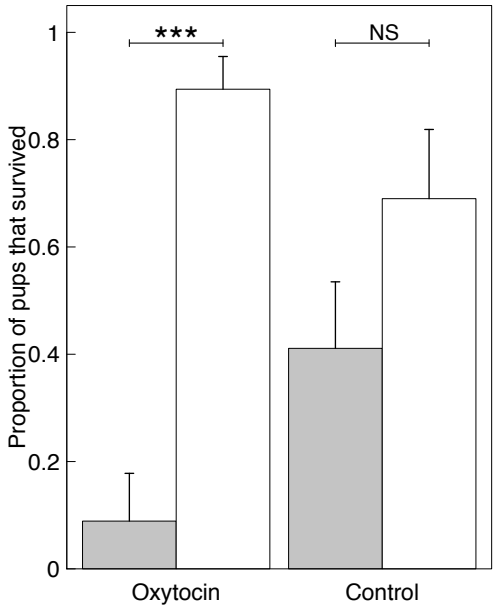

Figure 2. The proportion of pups that survived in the first communal litter only (both successful and unsuccessful communal litters), first-born (grey), and second-born (white) litters by treatment, given as mean ± SE, *** $P < 0.001$

We additionally examined the number of pups killed in the first-born litter of each communal litter (successful and unsuccessful) and found no significant effect of litter size of the first-born litter, litter size of the female about to give birth, or treatment.

Effect of Treatment on Litter Attributes

Pups born in the OT groups did not differ significantly in weaning weight from pups born in the CON groups (LMM: $\chi^2_1 = 1.82$, $P = 0.177$), tested as the weaning weight for each pup born (average weaning weight ± SE: OT: 10.7 ± 0.1 g and CON: 11.7 ± 0.1 g). Weaning

weight was not significantly affected by litter size at weaning or age difference between the litters. Furthermore, there was no significant effect of treatment on litter size at birth (average litter size at birth (all litters born); OT: 6.7 ± 0.4 pups and CON: 5.9 ± 0.3 pups; mean ± SE) or weaning (average litter size at weaning: OT: 4.0 ± 0.6 pups and CON: 3.1 ± 0.5 pups), with as increasing number of litters born to a female litter size increased significantly at birth (LMM: χ_1^2 = 7.85, P = 0.005) and weaning (LMM: χ_1^2 = 8.94, P = 0.003). This reflects the observation that primiparous females give birth to smaller litters than multiparous females (König & Markl, 1987).

DISCUSSION

In this study, we examined whether administration of peripheral OT influenced the ability of unfamiliar female pairs to cooperate through communal nursing. Our findings showed that pairs of females treated with OT, shortly before and during the initial phase of cohabitation, took significantly longer to form a successful communal litter in which both females weaned at least one offspring. On the one hand, this was surprising given that past studies investigating the role of OT on social and affiliative behaviours predict that individuals with elevated OT would be more likely to cooperate (De Dreu, 2012; Madden & Clutton-Brock, 2011; Wittig et al., 2014). On the other hand, our most recent study (Harrison et al., 2016) suggested that OT administered during first encounters (using the same approach as the current study) delays or lessens the formation of a preference for a previously unknown partner. Therefore, our results suggest that OT treatment delayed the tendency of female house mice to cooperate in the formation of a communal litter.

OT Delays Communal Nursing

Female house mice are generally social but aggression between pairs has been shown to impair cooperation (Rusu & Krackow, 2004), and competition over reproduction results in agonistic behaviour (König, 1994a; König & Lindholm, 2012; Palanza et al., 2005; Rusu & Krackow, 2004). In the current study, female pairs did not differ significantly in the number of days spent resting in side-by-side contact prior to parturition and aggression levels were low in both treatments. While we did not specifically monitor other female behaviours prior to parturition in this study, results from a previous study, Harrison et al. (2016) found, during an identical 3 days of cohabitation and treatment, no significant difference between OT- and CON-treated female pairs with regard to socio-positive and -negative behaviours. In only three pairs (two OT, one CON) did females exhibit aggression towards each other after birth of the first litters leading to separation due to incompatibility and were then considered unsuccessful. These observations, taken together with the findings of Harrison et al. (2016), provide little evidence to suggest that there was a difference between treatments with regard to affiliative or intolerant behaviour between the females. Furthermore, OT administration did not impact the females' ability to reproduce, as time until birth of first litters did not differ by treatment. Past research confirms this as mice whose OT receptor gene was inhibited were still able to function reproductively and gave birth (Russell & Douglas, 2003; Veening, de Jong, Waldinger, Korte, & Olivier, 2014).

Interestingly, OT females took longer to successfully wean a communal litter. This was not because OT females raised their litters solitarily (for example, by using the different cages accessible to the group), or because they differed in the number of communal litters attempted

(successful and unsuccessful combined) when compared against the CON. Females in the OT group did have potential communal litters as both females gave birth within 16 days of each other. Our results showed that this delay in cooperation was most apparent during the first communal nursing attempt, as more first-born litters failed (complete loss of pups) in the OT group than in the CON group. Accordingly, pup survival in this first attempt of a communal litter was significantly lower in the first-born OT litter than in the second-born, which was not the case in the CON litter, suggesting that OT litters suffered higher levels of initial pup mortality. Therefore, before another attempt at a communal litter could be made, females had to wait for the usual gestation time and birth intervals, which led to an increased latency of on average 31 days until a successful communal litter was established. This could suggest that OT females had a reduced propensity to cooperate through communal nursing.

Why did administration of OT during the first 3 days of cohabitation lead to increased pup mortality and delayed cooperation in female house mice? McCarthy et al. (1990) found that subcutaneous OT injection decreased infanticidal behaviour in virgin and pregnant female house mice when a pup was placed in their home cage. In contrast, other studies have shown that naturally elevated OT levels released during lactation and the onset of maternal behaviour were related to increased maternal aggression towards intruders postpartum (Bosch, 2013; Bosch & Neumann, 2012; Ferris et al., 1992). Given that in the current study the time between experimentally elevated OT and infanticidal behaviour was much longer than those in these previous studies, it may be less likely that OT acted directly on maternal aggression. Potentially, OT may have led to increased social recognition (Bartz et al., 2011; Bielsky & Young, 2004; Ferguson, Aldag, Insel, & Young, 2001; Ferguson et al., 2000) which could have reinforced recognition of the female's own pups. Again, this explanation would suggest that the OT manipulation worked over a long time, which is less likely. The initial higher infanticide observed among OT females here may therefore be linked to increased female competition (Palanza et al., 2005), or a reduced incentive to cooperate. This could suggest that the timing of OT administration in house mice may have different implications for infanticidal behaviour.

It has been suggested that OT effects are likely to be dependent on the characteristics of the situation and the interaction partners present (Bartz et al., 2011; Crockford et al., 2014; Wittig et al., 2014). Declerck and colleagues (2010) suggested that when appropriate social information about a partner was lacking OT could diminish cooperation. Additionally, if a partner was believed unreliable OT might lose its trust-enhancing effects (Mikolajczak et al., 2010). In our study, the first OT administration happened prior to any female interaction meaning that females had no prior social information on their new partner. In accordance with the suggestion by Declerck et al. (2010), we found a diminished propensity to cooperate among these females, which could indicate that the timing of administration was critical in determining how OT impacted communal nursing behaviours. Additionally, females treated with OT in an identical initial set-up demonstrated a lack of preference for their cohabitation partner relative to a novel partner (Harrison et al., 2016). In house mice, sharing a group with a preferred social partner is important for successful cooperation and associated with higher lifetime reproductive success than being experimentally grouped with a previously un-preferred partner (Weidt et al., 2008). In the present study, this lack of preference may have also occurred between the females treated with OT, and may not have allowed for the reinforcement of socio-positive behaviours, which could explain the delay in communal litter formation and high infanticide.

OT as Cause Versus Consequence in Partner Preference Formation

Beery and Zucker (2010) showed that OT was not required for initial formation of a preference in female meadow voles, *Microtus pennsylvanicus*, and Peñagarikano et al. (2015) found that OT-treated wild-type mice did not differ significantly from a control group in time spent interacting socially on initial encounter with a stranger. Therefore, assuming that increased circulating OT levels are not the cause but the consequence of interacting with preferred social partners (Crockford et al., 2013; Wittig et al., 2014), we hypothesize that its effects are time sensitive and partner specific. For example, OT levels were elevated in chimpanzee urine after grooming with a preferred partner (Crockford et al., 2013), blood OT levels increased in dogs, *Canis familiaris*, after short-term positive interactions with their owner (Handlin et al., 2011), and plasma OT levels increased in children after physical contact with their mothers (Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005). Therefore, an increase in OT induced by a preferred or familiar social partner could result in an increased propensity to cooperate only with that social partner. In our study, OT was administered prior to initial encounter with the other female so the elevated peripheral levels were not a result of social interactions with the new partner. This could mean the female did not consider this partner as "trustworthy" or a preferred partner.

In contrast, studies in humans have shown that OT may also become elevated after contact with a stranger or unfamiliar individual, since mothers had higher urine OT levels following interaction with an unfamiliar child than when interacting with their own biological child (Bick & Dozier, 2010). Additionally, Morhenn, Park, Piper and Zak (2008) found that circulating OT levels increased after receiving a massage but only when it was followed by an act of trust by a stranger. Plasma OT levels also increased in females who did not suffer from relationship anxiety following a relaxation massage given by a stranger (Turner, Altemus, Enos, Cooper, & McGuinness, 1999), thus indicating that some OT-induced responses may be species specific and potentially dependent on the social system being studied.

To better understand these concepts, future studies could test whether the effects of OT on partner preference and cooperation depend on familiarity by allowing previously unfamiliar and unrelated female mice to become familiar with each other before administering OT and then testing for a partner preference. In such a scenario, we would then predict that OT administration would result in improved cooperation among these familiar partners when compared to controls.

Influence of OT on Maternal Investment in Pups

We found no treatment effect on litter size at birth or weaning. In group-living female house mice uneven litter size at birth has been demonstrated to decrease the propensity of sisters to cooperate when they attempt to avoid exploitation from having the smaller litter (Ferrari et al., 2016). As we found no effect, it is unlikely that this was a contributing factor towards the decreased cooperation. We also found no effect of litter size of the first-born litter and of the female about to give birth on the number of pups killed in these first litters. Additionally, we found no effect of treatment or age difference between the litters on weaning weights of pups suggesting that females, although differing in the latency until formation of a successful communal litter, did not differ in their investment to the pups once present. This supports findings that suggest females invested in accordance with the combined communal litter size (Ferrari et al., 2015). Therefore, our previous findings on reduced partner preferences for a

cohabitation partner in OT-treated females (Harrison et al., 2016), combined with the current findings of increased infanticide, indicate that OT affects the propensity of females to cooperate.

Conclusions

Our findings suggest that administration of exogenous OT during the early stages of female relationships reduced their propensity to cooperate. Female treatment had no effect on the ability to reproduce, as we found no difference in time until birth of first litters. However, OT-treated females took longer to successfully establish and wean a communal litter, which was linked to the higher rates of pup mortality (via infanticide) in the first-born litter produced.

In sum, these findings add to the growing number of studies that have also found conflicting results in relation to the effect of OT on social and cooperative behaviour (Bales et al., 2013; Beery, 2015; Brosnan et al., 2015; Harrison et al., 2016; Peñagarikano et al., 2015), and support findings that suggest OT effects can be very varied and context or partner specific (Beery, 2015; Campbell, 2008; Donaldson & Young, 2008; Insel & Young, 2001). Future studies could investigate the mechanisms at play here. Our study does not support the hypothesis that OT acts to facilitate familiarization, and suggests that when administered in house mice during early social interactions with a stranger OT delays cooperation with that partner through communal nursing.

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Conclusions and Outlook

GENERAL DISCUSSION

The main aim of this thesis was to examine communal nursing decisions and social preferences in female house mice, by combining data collected from a free-living population of house mice with laboratory studies using wild-derived mice. Communal nursing in female house mice is a key example of cooperation, and laboratory studies suggest it to be a selected component of a female's reproductive behaviour. Studies assessing communal nursing behaviour have principally focused on the direct fitness benefits gained through sharing in the care of, and indiscriminately nursing own and non-offspring (Manning et al. 1995; Hayes 2000; König 2006). However, surprisingly little is known about communal nursing under free-living situations, where environmental conditions are unpredictable and the social environment is variable. Therefore, the decisions behind communal nursing, and the factors that drive a female to combine her litter with another female are poorly understood. Using detailed information obtained on individual communal nursing decisions we showed that females appeared choosy in their choice of communal nursing partner (chapter 1). In addition, using spatial genetic structure analysis we demonstrated that female house mice share their home area with individuals that are genetically similar to them, and within such a social environment females preferred to form communal litters with a female who had recently given birth. The strength of associations between a female and her preferred female partners, within her home area, was driven by pairwise relatedness, whereby females preferred to spend time with females that were more closely related to them (chapter 2). Female preferences were relatively stable over time, and those with fewer female social partners had preferences that were more stable, and weaned more litters within a six-month time period.

We further investigated whether oxytocin (OT) had an influence on initial social interactions between two unfamiliar female house mice, and whether it would facilitate the formation of a preference. OT has been implicated in the facilitation of social preferences, as well as the enhancement of cooperative and affiliative behaviours (Carter et al. 1992; Beery and Zucker 2010; Madden and Clutton-Brock 2011; Anacker and Beery 2013; Crockford et al. 2013; Mooney et al. 2014; Wittig et al. 2014; Carter and Wilkinson 2015). Nevertheless, despite OT ability to enhance positive social perceptions it can also increase negative ones (De Dreu et al. 2012; Crockford et al. 2014; Beery 2015). Our findings indicated no difference, neither in socio-positive nor socio-negative behaviours, when comparing OT-treated females against saline-treated controls, over a 3-day cohabitation period. However, when a female was presented with a choice between her cohabitation partner and a new unfamiliar female, OT-treated females demonstrated no preference for their previous partner, this was in contrast to the control-treated females. Therefore, increasing peripheral OT appeared not to be involved in initial encounters with a stranger, and may even have hindered the formation of a preference for this new partner (chapter 3). In a next step we tested the effect of OT on a female's willingness to cooperate through communal nursing. Here we found that OT-treated females took longer to successfully wean a communal litter, suggesting that OT delayed the tendency of female house mice to affiliate and cooperate in rearing a communal litter (chapter 4). Given the excitement surrounding OT and its 'love' hormone status, our findings were in contrast to expectations; nevertheless they contribute to the growing field of OT related studies and our knowledge of its effects.

Taken together, this thesis suggests that in communal nursing partnerships relatedness between females, alongside time spent together, are important factors in communal nursing decisions and partner preferences. The facilitation of these preferences, and the propensity of

female house mice to cooperate, however, appears not to be strengthened by peripheral administration of OT.

Communal Nursing Decisions and Social Preferences

Laboratory studies have shown that through communal nursing females benefit from increased lifetime reproductive success, especially if nursing with a related, familiar or preferred female partner (König 1994a; 1994b; Weidt et al. 2008), or in comparison to solitary nursing. Empirical studies have also highlighted the potential for exploitation during communal nursing, in particular when there is a disparity in litter sizes (Palanza et al. 2005; Ferrari et al. 2015; 2016; Schmidt et al. 2015). Therefore, choice of communal nursing partner should be important in order to maximise the benefits gained and reduce the exploitation incurred. The benefits gained from social preferences or choice of partner include increased offspring survival, increased longevity, decreased stress and increased group cohesion (König 1994a; Silk 2007; Yee et al. 2008; Shultz and Dunbar 2010; Silk et al. 2010; Seyfarth and Cheney 2012), and have been observed in a range of taxa of both kin and non-kin pairings. Therefore, the motivation to communally nurse is potentially driven by the fitness benefits obtained from sharing in the care of own and non-offspring. In the past, studies on female house mice have predominately focused on empirical findings where choice of partner was not assessed, and conditions were controlled and simplified. However, house mice across Europe occur in anthropogenic habitats, such as farm buildings and/or grain stores (Pocock et al. 2004), hence our study population living in an old barn is considered a natural habitat for house mice. Using data from our free-living population of house mice enabled us to assess communal nursing decisions and social preferences in the context of their natural environment, when exposed to natural selection, and gain a better understanding of the factors influencing female decisions under a less controlled, and more complex social setting.

Our initial aim was to verify the existence of preferences observed in laboratory studies (Weidt et al. 2008), and classify the factors that are important in a communal nursing partner. Interestingly, we found that females were 'choosy' when deciding to communally nurse, whereby they did not always communally nurse when they had the opportunity to do so. Focal females were more likely to form a communal litter with increasing number of potential partners available, which improved the chance that her preferred partner would be available. This supported previous findings from Weidt et al. (2014) who found similar results from the same population, but at a significantly lower population density, half the size of our study (during the period of our data analysis the maximal adult population density was 2.6 mice per square metre). Our results indicated that choice of partner was indeed an important aspect in communal nursing decisions, especially as laboratory studies showed that females benefit more when communally nursing with a partner they prefer (Weidt et al. 2008).

A significant finding was that female decisions were carried out in a social environment that consisted of genetically similar females, and within such an environment females chose partners that had recently given birth (chapter 1). We further found that the strength and duration of female dyadic relationships were driven by pairwise relatedness, whereby females associated more often with females that were more closely related to them (chapter 2). Female preferences were stable over several months, and when there were fewer females in a social group, stability of these preferences was higher. These findings compliment the suggestion that female house mice are the philopatric sex (Gerlach 1990; Dobson et al. 2000; Rusu and Krackow 2004), whereby dispersal is limited and social interactions are more common among related conspecifics (Hamilton 1964a; 1964b; 1970). Our results also support those of studies

that have observed associations in laboratory enclosures where females formed spatial associations and cohabited more often with sisters (Dobson et al. 2000; Rusu and Krackow 2004). According to kin selection theory the evolution of costly cooperation is facilitated when interacting individuals are related (Hamilton 1964a; 1964b; 1970; Taylor 1992; West et al. 2001; Queller 2011), and the potential direct fitness benefits lost from reproductive competition between females (as for example through infanticide) would likely be compensated for by the indirect benefits gained from nursing with a related partner (Hamilton 1964a; 1964b; Mumme et al. 1983). In addition, the costs obtained through exploitation are more frequently tolerated when a partner is related (West, et al. 2002; Mathot and Giraldeau 2010; Ferrari et al. 2016), and when groups are composed of related females the investment into unrelated young can be reduced (Manning et al. 1992; 1995), this could explain why females invest in communal nursing despite the associated costs or risks. Additionally, females could also use alternate cues when assessing the suitability of her available partners, such as major histocompatibility complex gene (MHC) or major urinary protein genotype (MUP) similarities. While previous studies have shown partner discrimination based on MHC or MUP genes in mice (Manning et al. 1992; Roberts and Gosling 2002; Green et al. 2015), in relation to communal nursing decisions such discrimination remains to be tested.

Females additionally preferred to form a communal litter with a partner that had recently given birth; such a decision could indicate reproductive synchronisation between the females (Agrell et al. 1998). In animal societies it is believed that selection should favour females who synchronise reproduction, or give birth later than other group members, especially when infanticide is common (Agrell et al. 1998; Riehl 2016). Breeding synchrony is beneficial to females as those with new-born pups are least likely to perform infanticide (Soroker and Terkel 1988), suggesting that if females synchronise breeding their investment should be focused on offspring care rather than infanticide (Agrell et al. 1998). Female house mice use infanticide as a manipulative tactic to guide their partners attention towards caring for non-offspring (mainly through providing a larger share of milk to the partner's offspring) and to lengthen exploitation (Ferrari et al. 2015; 2016; Schmidt et al. 2015). This would suggest that females should prefer a partner with a younger litter in order to minimise infanticide or escalating conflicts, and that unlike in laboratory studies (Weidt 2007), females in our free-living population may try to synchronise reproduction. Therefore, choosing a female with a younger litter could imply synchronisation, and in a scenario where there are multiple options available it may be easier to implement, which in turn may explain why under some situations females did not communally nurse. A larger age disparity between litters can also lead to an increased risk of discrimination by the other female. In laboratory mice, Hager and Johnstone (2005) observed that offspring gained more weight when nursed by their own mother than littermates that were cross-fostered at 15 days old until weaning. Such preferential investment in own offspring would be in the mother's interest, but not in the interest of the cooperating partner, creating a conflict over investment into the communal litter. Other studies further report reduced pup mortality and increased competition between the pups with increasing age disparity (Svare and Gandelman 1973; Mennella et al. 1990; Manning et al. 1995; Sugawara et al. 2012; Schmidt et al. 2015; Tučková et al. 2016). Smaller age differences between litters could therefore be beneficial to stabilise cooperation and to improve pup survival. However, whether reproductive success in a free-living population is equivalent to laboratory findings is currently being quantified, and with these results we will better understand the implications of communal nursing in such an environment.

Empirical studies have further shown litter size to play a role in the decision to communally nurse, whereby females will avoid joining another female when the partners litter

size is larger, due to the increased risk of being exploited (Ferrari et al. 2016). However, litter size at birth in our free-living population is difficult to accurately quantify. Females consider the opening of nest boxes fairly invasive and in such an event may move litters to another nest (pers. obs.), which could result in mortality or misplacement of pups. Therefore, in order to avoid disturbances we do not routinely search for litters on a daily basis, meaning pups are often found when they are already a few days old, at which point infanticide by conspecifics may already have occurred. Therefore, despite finding no support for litter size influencing communal nursing decisions, we cannot completely rule it out as a potential deciding factor. Use of semi-natural enclosures in the laboratory, would allow litter size at birth to be more accurately measured and permit testing of such predictions on choice of communal nursing partner.

Oxytocin - Social Preference and Communal Nursing

In the past studies have indicated that time spent in close association can define social preferences among individuals within a social group or during a preference test (Carter et al. 1992; Williams et al. 1994; Insel and Hulihan 1995; Silk et al. 2003; Bielsky and Young 2004; Weidt et al. 2008; Cameron et al. 2009; Schülke et al. 2010). Therefore, we could assume that female mice may establish a social preference for another female through time spent interacting, prior to communal nursing, the finding in chapter 2 compliments such a suggestion, as females associated more often with their top partners.

OT has been implicated in the facilitation of sociability and the motivation of individuals to affiliate more generally (Campbell 2008). Therefore, we tested whether OT administered during a period of cohabitation would facilitate a social preference between pairs of unfamiliar female house mice. The majority of attention surrounding OT has focused on the positive effects it can induce, however, it has been shown to enhance both positive and negative social perceptions (De Dreu 2012; Crockford et al. 2014; Beery 2015). Results from our study support such ideas, as OT, when administered at the onset of establishing a relationship, did not increase social interactions between pairs of unfamiliar females, beyond that of what we would expect naturally (as demonstrated by the control-treated females, chapter 3). Our results, taken together with other findings, suggest that elevated endogenous OT is not a facilitator of social positive interactions between unfamiliar female house mice. Despite finding no difference in initial social behaviours by treatment, we did find a steady increase in socio-positive behaviours across the 3 days, suggesting all female pairs became more affiliated. This increase in positive behaviours further indicates that time spent together could lead to social preference formation. It was only when OT females were presented with a novel, unknown female that they appeared to become choosier, suggesting that maybe OT increased social approach behaviour (Lim and Young 2006), or salience of social stimuli (Young and Barrett 2015). However, within our experiment it appears that OT acted to hinder the formation of a preference.

Some alternative suggestions to explain why OT-treated females spent a similar amount of time with both stimulus females during the preference test could be that OT acted as a social buffer leading to increased interest in the new stimulus female. Studies have demonstrated that animals treated with OT showed increased social contact (Witt et al. 1990; Carter et al. 1992), and increased exploratory behaviours (Dharmadhikari et al. 1997). OT manipulation was further demonstrated to prevent stress induced social avoidance in rats, and has facilitated a social preference for novel conspecifics in both mice and rats (Windle et al. 1997). Consequently, with the information from these studies, we could hypothesize that OT enhanced the female's

exploratory behaviour during the novel environment of the preference test. However, we did not explicitly test for these behaviours and therefore, can only speculate on such ideas.

Social preferences appear to be important for female house mice reproductive success, and when females are kept in groups of un-related females, up to 75% of them will form a significant association with one other female (Weidt et al. 2008). However, we found that OT females took longer to successfully wean a communal litter, suggesting that OT delayed the tendency of female house mice to affiliate and cooperate in rearing a communal litter (chapter 4). Studies have implied that OT effects are likely dependent on the characteristics of the situation and the presence of specific interaction partners (Bartz et al. 2010; Crockford et al. 2014; Wittig et al. 2014). OT can also lose its trust-enhancing effects when a partner is believed unreliable (Mikolajczak et al. 2010), and can even reduce cooperation when appropriate social information is lacking (Declerck et al. 2010). In our study, OT was administered prior to the females having any social information about one another, and the elevated OT was not a result of positive social interactions between the two females. Therefore, we suggest that administration of exogenous OT during the early stages of the female's social relationships may have later implications on their willingness to cooperate. We found a diminished propensity to cooperate among OT-treated females; thus we propose that the timing of OT administration may be important.

We hypothesize that OT effects may be influenced by interactions with a preferred social partner, and may not act as an initiator of social interactions (see also: Crockford et al. 2013; Wittig et al. 2014). Social bonds and positive social interactions are often characterised by repeated physical contact and positive social stimuli between individuals (Uvnas-Moberg 1998). OT therefore is likely associated with the enhancement or facilitation of an already established bond. Furthermore, OT may potentially stimulate its own release through a positive feedback loop and activation of own receptors (Ludwig and Leng 2006). In our study, the initial boost of OT provided by the injection appears not to have played a role in early social interactions between unfamiliar female pairs, suggesting that prior familiarity may be important. We therefore suggest that the effect of OT was dependent on the interaction partner present, and the timing of administration. Studies have shown OT levels to be elevated after positive social interactions, such as food sharing with a conspecific (Wittig et al. 2014), grooming with a preferred partner (Crockford et al. 2013), or after a dog experienced positive interactions from their owner (Handlin et al. 2011). This could imply that an increased propensity to cooperate with a specific partner may only be as a result of elevated OT induced by a familiar or preferred partner. Despite this, some human studies have found that peripheral OT levels were elevated after interactions with a stranger (Turner et al. 1999; Morhenn et al. 2008; Bick and Dozier 2010). Thus, highlighting that an OT-induced response may be species specific, and potentially dependent on the social system. To better understand such concepts future studies could test whether OT effects are dependent on previous familiarity between females.

Conclusions

This thesis has described communal nursing decisions in female house mice by examining the factors that influence choice of communal nursing partner, social preferences and their stability between female partners. We further assessed the role of OT in facilitating social preferences or social bonds, and thus improving cooperation during communal nursing. Our findings contradict the common misconception that communal nursing is a consequence of sharing the same nesting sites. We demonstrated that female house mice have the capacity to choose a partner from a range of available options and develop preferences, within a socially

complex and spatially genetic home area. Since OT was demonstrated to hinder the formation of a social preference, and delayed successful communal nursing, these studies do not support the hypothesis that OT acts as a mechanism to facilitate positive interactions and preference formation. However, we are currently unable to definitively conclude that OT has either a negative or positive effect on social interactions and communal nursing behaviour in house mice. Taken together, our findings could suggest females use a hierarchy of cues when choosing a communal nursing partner, whereby a female simultaneously perceives different cues. Thus, as has been suggested in female mate choice decisions (Roberts and Gosling 2003; Mays and Hill 2004), a female's decision may rely on the relative fitness benefits that would result from choosing a high quality partner given the pool of partners available. Consequently, many questions about the factors influencing social preferences, communal nursing decisions, and the role of OT in these, remain unanswered. In sum, using the knowledge obtained from this thesis, we have shown that predictions from laboratory studies are not necessarily consistent across taxa, or comparable to wild populations. They highlight the importance of multiple testing, and the verification of findings, using both empirical and free-living studies. Importantly they provide a foundation for future studies to expand on our knowledge of communal nursing decisions and their implications.

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R4All. A Recipe for Success in R	1 ECTS
Conception and Statistical Analysis of Biological Experiments	3 ECTS
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